THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 120 DECEMBER 1, 1937 No. 4

THE RELATION OF THE HYPOPHYSIS AND ADRENAL CORTEX TO THE REMOVAL OF EXCESS GLUCOSE FROM THE BLOOD OF RATS

LEO T. SAMUELS, HERMANN F. SCHOTT AND HOWARD A. BALL[1]

WITH THE TECHNICAL ASSISTANCE OF M. L. LIPKIS[2] AND M. CHERNOW[2]

From the Departments of Pharmacology and Pathology, respectively, University of Southern California School of Medicine and the College of Medical Evangelists

Received for publication June 1, 1937

When glucose is given parenterally to fasted hypophysectomized rats the rate of removal of the excess sugar from the blood stream decreases with lapse of time after operation until it reaches a minimum in about five weeks (Samuels and Ball, 1937). Biasotti (1934) observed this diminished removal of excess sugar in hypophysectomized dogs. The progressive nature of this deficiency led us to postulate that the effect was not due to the direct rôle played by the pituitary secretion in the immediate process of glycogen storage. An indirect effect such as atrophy of the adrenals, or interference with the normal metabolic processes of the liver cells seemed probable. Leloir (1934) reported a decreased glucose tolerance in adrenalectomized animals, and showed this to be due to a decreased rate of glycogen formation in the liver. Britton and Silvette (1934) also reported a decreased rate of glycogen formation and abnormally high blood sugars when glucose was given to adrenal-less rats. The latter authors, and Buell, Anderson and Strauss (1936), found a reduced synthesis of liver glycogen from d-lactic acid in such animals. Evans (1936) found that the adrenal played a part in the reduced carbohydrate levels of hypophysectomized animals. This evidence led us to investigate the rôle of the adrenals in the decreased glucose tolerance of our animals.

If the effect of removal of the hypophysis on the glucose tolerance is due simply to the subsequent atrophy of the adrenals, hypophysectomized

[1] Aided by grants from the American Association for the Advancement of Science and the Committee on Scientific Research of the American Medical Association.

[2] On N. Y. A. appointment.

animals should show less deficiency than completely adrenalectomized animals. Furthermore, the sugar tolerance curves of hypophysectomized-adrenalectomized animals should parallel those of the adrenalectomized group, and the effects of injections of extract should be similar in all three cases. Experiments herewith described revealed that adrenal atrophy is but a minor factor, at most, in the alteration of the blood sugar curves.

METHODS. Male rats 4 to 6 months of age were used. All came from the same basic stock though obtained from two different sources. Hypophysectomy was performed by the neck approach. In the earlier operations the naso-pharynx was opened; later this was avoided. The usual technic of adrenalectomy was employed through a single lumbar incision. The completeness of both operations was checked by careful post-mortem dissection with the aid of a loupe and any suspicious tissue was examined microscopically. Completeness of adrenalectomy was checked also by placing 40 operated animals on tap water and stock diet. Thirty-seven died within seventeen days.

Injections of 50 per cent glucose were made into the saphenous vein in the unanesthetized animal in the dose of 0.125 gram per 100 grams of body weight. To obtain blood the tail was warmed with radiant heat, the tip clipped with shears, and the sample pipetted directly after discarding the first few drops. When heart samples were secured a small needle was sealed on the end of the micro-pipette and slight suction applied through a rubber tube and mouthpiece. Samples were ordinarily taken at intervals of 10, 20, 30, 60 and 120 minutes after glucose injections. These were 0.025 cc. in amount and were added to 5 cc. of tungstic acid for deproteinization. The centrifugates were analysed by the Folin micro-method.

The blood sugar curves were observed seventeen days after hypophysectomy and, if repeated on the same animals under different conditions, again on the 21st day. Adrenalectomies were performed twenty-four hours, four days, and two weeks before testing.

The adrenal cortex extract used was the product of the Wilson Laboratories purchased on the open market.

RESULTS. *Hypophysectomy.* Table 1 shows the average blood sugar curves for hypophysectomized and normal animals after intravenous injection of glucose. The values indicate the same inability of the hypophysectomized animals to remove excess sugar from the blood that was demonstrated when other routes of sugar administration were employed (Samuels and Ball, 1937). Thus the drop in blood sugar in the normal animals within the first half hour was much more rapid than in the hypophysectomized group, and was lower even at the ten minute interval. During the second hour, however, the blood sugar level of the hypophysec-

tomized animals dropped more rapidly reaching the normal level by the 120 minute period.

Analyses of the bladder contents were made on a few animals of both groups but the reducing matter present was insignificant when compared with the amount of sugar which disappeared from the blood.

The possibility that the difference in tolerance might be due to circulatory changes was checked in two ways. Red cell counts were made on animals of both groups at the same time that blood sugar samples were taken. These showed no significant difference. Simultaneous heart-blood and tail-blood samples were also analyzed. The glucose in the heart blood was somewhat higher than the peripheral blood at the start (5 to 9 mgm.) and somewhat lower at the end (9 to 25 mgm.). This was particularly apparent in the hypophysectomized animals. The differences between the glucose tolerance curves of the normal and hypophysectomized rats would be even more striking if compared on the basis of heart blood samples.

TABLE 1

Average blood sugar curves of normal and hypophysectomized male rats after intravenous injection of 0.125 gram glucose per 100 grams body weight

GROUP	NUMBER OF ANIMALS	TIME AFTER INJECTION OF GLUCOSE					
		Fasting	10 min.	20 min.	30 min.	60 min.	120 min.
		mgm. glucose per 100 cc. blood					
Hypophysectomized	25	73±1.5	303±5.5	268±4.5	241±4.9	163±6.0	98±3.7
Normals	24	81±1.7	272±4.7	208±2.7	161±2.7	119±1.8	97±1.4

Effect of adrenal cortex extract after adrenalectomy and hypophysectomy. The glucose tolerance curve for adrenalectomized animals on tap water was somewhat elevated five days following operation. Adrenal cortex extract was given to a group of these animals subcutaneously in 0.2 cc. doses twice daily for three days and once on the following morning just before determining the sugar tolerance. The curve not only was normal, but by the end of one hour fell significantly lower. On the other hand similar administration of the extract to hypophysectomized rats produced no change from the curve established for such animals when not injected. Figure 1 shows the average curves for the various groups. The differences between points on the contrasting curves were shown to be statistically significant.

Effect of drinking Rubin-Krick salt solution. The substitution of the salt solution of Rubin and Krick (1933) for the tap water in the drinking bottles did not increase the sugar tolerance of hypophysectomized rats tested 17 days after removal of the gland. As expected, adrenalectomized

rats, on the Rubin and Krick solution, showed a normal sugar tolerance when tested at the usual 5 day period after operation, in contrast to the reduced tolerance of similar rats on tap water.

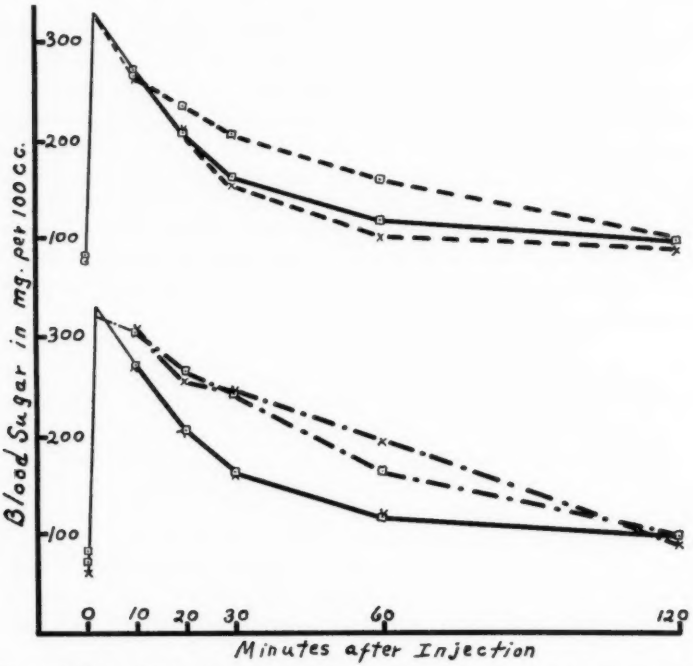


Fig. 1. Effect of injection of adrenal cortex extract on the intravenous glucose tolerance curves of normal, adrenalectomized, and hypophysectomized male rats. ——— normal curves; ---------- adrenalectomized animals; ·—·—·— hypophysectomized animals. The points marked X signify values found following cortin injection; ⊡ values without cortex extract.

The light lines indicate the estimated course of the blood sugar in the first 10 minutes, assuming that diffusion equilibrium is established between blood and tissues within the first two minutes. The peak is determined by adding 250 mgm. per 100 cc. to the fasting value, based upon the findings of Cori (see text).

The curve for the normal animals injected with cortin (lower) corresponds so closely to the uninjected group that the crosses have been indicated as lying partially covered by the squares, and no second curve has been drawn.

When adrenalectomized rats on Rubin and Krick solution were tested on the fourteenth day after operation, their composite sugar tolerance curve was similar to that of the five day animals on tap water. Most of the latter died before the fourteenth day.

Hypophysectomy-adrenalectomy. Removal of the adrenals from previously hypophysectomized rats did not further decrease sugar tolerance, in spite of marked shortening of the survival time. Animals with both the pituitary and adrenal glands removed died much more quickly when kept on the stock ration than rats deprived of either gland alone, even though the former were given cortex extract during the first three days after adrenalectomy to permit recovery. These animals also died so uniformly when fasted 24 hours that this period had to be reduced to 12 hours or less. Apparently some of the metabolic effects of both organs are additive, but not those affecting the glucose tolerance curve.

Influence of fasting. At first it appeared that the differences between the sugar tolerance curves of hypophysectomized and normal animals might be related to the rapid fall in blood sugar and liver glycogen on

TABLE 2

Effect of fasting on the glucose tolerance curve of hypophysectomized male rats

GROUP	NUMBER OF ANIMALS	TIME AFTER INJECTION OF 0.125 GRAM GLUCOSE PER 100 GRAMS BODY WEIGHT					
		Before	10 min.	20 min.	30 min.	60 min.	120 min.
		mgm. glucose per 100 cc. blood					
Hypophysectomized 17 days, not fasted	8	114	357	318	247	168	124
Normals, not fasted	8	125	285	183	151	142	124
Hypophysectomized* 21 days, fasted 12 hr	7	80	301	275	258	162	85
Normals,* fasted 12 hr	8	116	277	212	177	125	111

* These are the same groups that were used in the unfasted experiment.

fasting which was described by Russell and Bennett (1937). The data in table 2 do not indicate any such relation. The same rats were used in each experiment. The curves on the animals when fasted one hour were obtained seventeen days after hypophysectomy; those after a 12-hour fast were obtained twenty-one days after operation. Since the longer period after hypophysectomy would tend to decrease the tolerance it should have accentuated the effect of fasting. Instead the only differences between the two series of data on the hypophysectomized group were that the blood sugar values of the animals when fasted 12 hours started at a lower level and fell more rapidly at the end. The intermediate values agreed closely. Similar curves on a parallel group of normal animals showed a less rapid drop during the first thirty minutes after injection following the 12 hour fast than when the animals were fasted one hour. The low fasting blood sugar and the low level of the curve for fasting hypophysectomized rats

two hours after injection of sugar is characteristic of the loss of pituitary function (Russell and Bennett, 1937).

Discussion. a. *Significance of alterations in the sugar tolerance following hypophysectomy.* Three major processes are concerned in the immediate removal of excess glucose from the blood stream: 1, diffusion into the tissues; 2, formation of glycogen; 3, utilization as fuel.

Diffusion takes place rapidly in normal animals. Where equilibrium between blood and tissues had been attained through diffusion, Cori (1931) found that the total sugar in rats was equal to the blood-sugar level times half the body weight. Since the amount of glucose given our animals was 0.125 gram per 100 grams of body weight the calculations of Cori would indicate that a rise in the blood sugar to 250 mgm. per cent above the initial level would be expected, providing no other factor had removed appreciable amounts of sugar in the meantime. In all of our data the 10 minute averages were at this level or below. The disturbances observed in the glucose tolerance curves were not due then to delayed diffusion.

Thus the changes seen are probably related to glycogenesis and glucose catabolism. Glycogen storage is a rapid and variable process dependent largely on the level of the blood sugar while utilization of sugar by the tissues is primarily dependent on the activity of the animal and has little to do with the blood sugar level. In our experiments the animals were in small individual cages and remained relatively quiet. Since, therefore, the utilization factor was probably fairly constant throughout the two hour period, we can infer that the decreased disappearance of glucose seen in hypophysectomized rats during the first half hour when the sugar level was high must have been due to disturbances in glycogenesis. Since the liver is the principal factor in the rapid removal of large amounts of glucose from the blood (Soskin et al., 1937) it would seem that this is the primary site of reduced function.

On the other hand, during the second hour the greater part of the excess glucose has already been removed and the rate of utilization becomes presumably the major factor in determining the curve. During this period the slope of the curve for the hypophysectomized animals is greater than that of the normals. This evidence for an increased rate of utilization in the hypophysectomized group agrees with the observations of Greeley (1935) and Russell and Bennett (1937).

b. *Significance of circulatory and renal factors.* We have mentioned the insignificant loss of sugar in the urine under the conditions of this experiment. Also, red cell counts have indicated that no significant hemoconcentration occurred in the hypophysectomized rats. We have made no measurements of blood pressure in these animals, but Braun-Menendez (1932) found in hypophysectomized dogs that the pressures were either normal or only slightly decreased. Our gross observations pointed in

this direction since blood continued to flow through the saphenous vein while glucose solution was being injected at a fairly rapid rate; but in the adrenalectomized and hypophysectomized-adrenalectomized animals the blood flow was entirely stopped by similar injections, and the rate had to be decreased to avoid mortality. Yet, in all the groups where both heart and tail blood samples were taken there was evidence of good distribution of the sugar. We believe, then, that while circulatory changes have not been entirely ruled out as a major factor, the preponderance of evidence is against such an explanation.

c. *The adrenal factor.* Pituitary removal appears not to affect the sugar tolerance by way of the adrenals, since in our experiments alterations of adrenal effect (gland removal, injections of adrenal cortex extract, electrolyte feeding) did not influence the glucose tolerance curve of the hypophysectomized rats. On the other hand, there is the possibility that the adrenal may exert its influence on the curve by way of the hypophysis, but our experiments were not planned to cover this point.

The return to normal tolerance shown in adrenalectomized rats when given cortex extract was apparently due to the replacement of the adrenal deficiency since administration of extract to normal animals did not affect their tolerance. That the extract benefited sugar tolerance through improvement in water and salt metabolism is suggested by the delay in glucose tolerance impairment when the Rubin and Krick solution was given to adrenalectomized rats.

d. *The effect of fasting.* Russell and Bennett (1937) found that glycogen disappeared from the liver and the blood sugar fell much more rapidly in hypophysectomized animals during fasting than in normals. Since our studies were largely on animals fasted 24 hours the reduced rate of storage which we observed in the absence of the pituitary might be a secondary result of the disturbed metabolism of the cells concerned due to low carbohydrate levels. The comparison of the same animals fasted 1 hour and 12 hours however indicates that the deficiency in glucose removal from the blood is one which progressively develops with the continued absence of pituitary secretions, and is not dependent on immediate changes in metabolism.

CONCLUSIONS

1. The decreased ability of hypophysectomized rats to remove excess glucose from the blood is not primarily dependent on the adrenal cortex, fasting, circulatory depression or urinary loss.

2. While not influencing the glucose tolerance, removal of adrenals in hypophysectomized rats introduces further disturbances in metabolism which decrease the length of life on fasting considerably below that of either the hypophysectomized or bilaterally adrenalectomized animals.

The metabolic influences of the pituitary, then, cannot be solely through the adrenal.

3. The rate of glucose storage appears to be much reduced in hypophysectomized rats.

4. Tissue utilization of glucose seems to be increased in the rat without pituitary.

REFERENCES

BIASOTTI, A. Compt. rend. Soc. de biol. **117:** 54, 1934.

BRAUN-MENENDEZ, E. Compt. rend. Soc. de biol. **11:** 477, 1932.

BRITTON, S. W. AND H. SILVETTE. This Journal **107:** 190, 1934.

BUELL, M. V., I. A. ANDERSON AND M. B. STRAUSS. This Journal **116:** 274, 1936.

CORI, C. F. Physiol. Rev. **11:** 143, 1931.

EVANS, G. This Journal **114:** 297, 1936.

GREELEY, P. O. Proc. Soc. Exper. Biol. and Med. **32:** 1070, 1935.

LELOIR, L. F. Suprarenales y Metabolism de los Hidratos de Carbono. Buenos Aires **10:** 216, 1934.

RUBIN, M. I. AND E. T. KRICK. Proc. Soc. Exper. Biol. and Med. **35:** 228, 1933.

RUSSELL, J. A. AND L. L. BENNETT. This Journal **118:** 196, 1937.

SAMUELS, L. T. AND H. A. BALL. Endocrinol. **21:** 380, 1937.

SOSKIN, S., H. E. ESSEX, J. F. HERRICK AND F. C. MANN. Proc. Amer. Physiol. Soc. 1937, p. 151.

THE EFFECT OF ATROPINE ON GASTRIC SECRETION AND ITS RELATION TO THE GASTRIN THEORY

JOHN S. GRAY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Received for publication June 7, 1937

Sacks, Ivy, Burgess and Vandolah (1) have reported that the only constituent of their pyloric extracts which stimulated gastric secretion was histamine, a fact which suggested the identity of histamine with gastrin. However, these authors and others (3) have pointed out a serious criticism of this conclusion. Atropine is able to abolish completely the secretory response of a Pavlov pouch dog to a meal, this secretory response presumably being accomplished through participation of gastrin. Yet atropine is unable to abolish the response of the gastric glands to a single subcutaneous injection of histamine. The authors therefore concluded that *a*, atropine prevents the formation of gastrin, or *b*, gastrin is not histamine and therefore no one has ever obtained this hormone in their extracts.

It was the purpose of this study to reinvestigate the action of atropine on gastric secretion in order either to establish or disprove the basis of the above criticism. It was felt that the proof was not yet conclusive because no careful comparison has been made of the effectiveness of atropine against a meal and against a histamine stimulus *of equal potency*. Furthermore, since the literature is in some disagreement as to the value of atropine in inhibiting gastric secretion, it was of interest to apply to this problem the method (2) recently devised in this laboratory for quantitatively estimating gastric inhibition.

METHODS. Five dogs with pouches of the entire stomach (vagotomized) were used throughout this study. These pouches were prepared from three months to two years before this work was begun.

The method employed for estimating inhibition was that described in a previous publication (2). It consists of maintaining a uniform level of gastric secretion of a standard rate (5 mgm. HCl per minute) by injecting histamine subcutaneously every ten minutes in a constant dose for the duration of the experiment. Having established and maintained this level of secretion for at least one hour (control hour) the drug to be tested is injected. Its effect on the secretion during the succeeding two hours is determined and expressed as percentage inhibition of HCl. Since in the

present experiments the atropine was injected subcutaneously and therefore its effect was manifested slowly, the two hour period was begun twenty minutes after the injection of atropine. The effect of various doses of atropine sulphate was studied as well as the effect of 1 mgm. of atropine at various control secretory rates.

In order to determine the effect of atropine on the secretory response to a meal, the animals were fed 200 grams of pork liver brought to a boil in 150 cc. of water. The dogs took from 15 minutes to one hour to consume the meal, since it directly entered the intestine.

The gastric juice was collected in twenty minute samples and titrated in toto with N/10 NaOH using Topfer's and phenolphthalein as indicators.

TABLE 1

Effect of atropine on continuous histamine secretion

	DOG 1		DOG 2		DOG 3		DOG 4		DOG 5		AVERAGE OF ALL DOGS	
ATROPINE	Control hour. HCl per minute	2 hours after. HCl inhibition	Control hour. HCl per minute	2 hours after. HCl inhibition	Control hour. HCl per minute	2 hours after. HCl inhibition	Control hour. HCl per minute	2 hours after. HCl inhibition	Control hour. HCl per minute	2 hours after. HCl inhibition	Control hour. HCl per minute	2 hours after. HCl inhibition
mgm.	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
1	3.44	58	1.94	48	2.10	60*	2.41	62	1.47	61	2.27	58
1			2.54	39								
1	4.40	53	4.41	38	4.64	30	4.54	46	5.91	37	4.97	37
1	6.37	47*	5.78	27*	6.02	23*	5.48	29	6.49	27*	6.03	31
1	8.50	23*										
1	9.57	13*										
0.5	5.01	25*	4.49	17*	4.78	25	5.66	23	5.22	29	5.03	24
1.0	4.40	53	4.41	38	4.64	30	5.48	29	5.91	37	4.97	37
2.0	5.30	58	4.93	41*	5.15	36	5.95	35	6.68	45	5.60	43

* Atropine increased gastric acidity in these cases.

RESULTS. The data concerning the inhibitory effect of atropine on continuous histamine secretion are shown in table 1. When the control rate of secretion is approximately 5 mgm. of HCl per minute 0.5 mgm. of atropine sulphate administered subcutaneously produces an average of 24 per cent inhibition; 1 mgm. produces 37 per cent inhibition, and 2 mgm. over 43 per cent. Also, as the control rate of secretion rises from 2.27 mgm. to 6.03 mgm. HCl per minute the degree of inhibition produced by 1 mgm. of atropine falls from 58 per cent to 31 per cent. Between histamine and atropine there is a mutual antagonism such as has been previously reported in the case of histamine and enterogastrone (2). However, the maximum effect obtained with atropine is only 60 per cent. Doses larger

than 2 mgm. have not been employed, because even this dose shows slight toxicity as indicated by restlessness and panting. However, 60 per cent inhibition seems to be the maximum effect obtainable against even the lowest rates of continuous histamine secretion. Thus, there exists a definite limit to the extent to which atropine will inhibit the effect of histamine on the parietal cells.

This situation does not obtain, however, in regard to the antagonism between atropine and a meal stimulus. This is illustrated in figure 1. Each of these curves is the average of four experiments, one on each of 4 dogs, all of which gave the same results. The latent periods, or the time

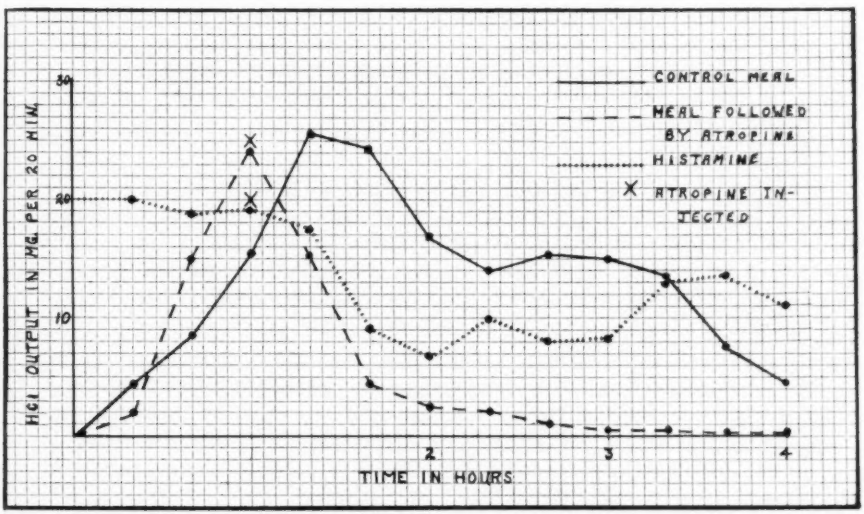


Fig. 1

elapsed between the beginning of the meal and the first appearance of free acid, averaged one hour, although it varied in different animals from twenty minutes to one hour and forty minutes. In order to prevent this variable from distorting the average curves, the first sample in which free acid appeared was taken as the initial value for the individual secretory curves which were then averaged. For the same reason the atropine was administered in each case one hour after the first appearance of free acid.

It should be noted in figure 1, that 1 mgm. of atropine subcutaneously completely abolished gastric secretion after a meal. In fact free acid disappeared in all four dogs within 40 minutes to 1 hour and 40 minutes after atropine was injected. This graph also shows that a continuous

histamine secretion of a rate somewhat less than the maximum rate reached after a meal, is not abolished by 1 mgm. of atropine. This shows conclusively that the ineffectiveness of atropine against histamine as contrasted to its effectiveness against a meal can not be interpreted on the basis of unequal rates of secretion. This phenomenon must be due to a qualitative difference in the two types of gastric stimulants.

The acidity of the gastric juice was decreased by atropine in the case of the meal. However, it was noted that atropine occasionally increased the acidity of the histamine secretion even though the total acid production was decreased. The instances in which this occurred are designated in table 1 by asterisks.

Discussion. The results show that atropine can produce a definite inhibition of histamine secretion, although under no conditions was there a complete abolition of secretion. Keeton, Luckhardt and Koch (3) and Klein (4) found in Pavlov pouch dogs that atropine gave only a slight inhibition of secretion stimulated by a single subcutaneous injection of histamine. Polland (5) observed the same in human subjects. We have observed with enterogastrone (2) that a greater degree of inhibition is obtained against continuous histamine secretion than against a secretion resulting from a single injection of histamine, even though the same quantity of juice was secreted during an hour period by both methods of stimulation. The quantitative difference between our results with atropine and those of the above investigators is therefore due to the different methods employed. We believe that our method gives not only more accurate results, but also truer ones, because the continuous type of secretion resulting from a low concentration of histamine in the cells acting over a long period approximates normal activity much closer than a secretion resulting from a high concentration of histamine acting over too short a period to produce its maximal effect.

It was noted that atropine might increase the acidity of the gastric juice while at the same time decreasing the total production of hydrochloric acid. Keeton, Luckhardt and Koch (3) in dogs, and Polland (5) in humans made the same observation. This is probably due to the fact that atropine can inhibit the formation of the non-acid as well as the acid elements of gastric secretion. If the non-acid elements (mucus, diluting or neutralizing fluids) are inhibited to a greater extent than the parietal secretion, a rise in acidity would be explained.

All investigators are not agreed as to the exact effect of atropine on gastric secretion in human subjects (see reviews by Altshuler (13) and Klein (4)). However, if those experiments are excluded in which histamine is used as the gastric excitant, the majority of workers have found a definite although incomplete inhibition of the rate of production of HCl accompanied by a decline in acidity. Perhaps the inconsistencies in this

work may be explained on the basis of the following considerations: *a*, differences in atropine dosage; *b*, differences in time at which the atropine was given in relation to the test meal; *c*, differences in the duration of the test period; *d*, differences in criteria used for determining inhibition (acidity, total acid production), and *e*, inability to control extra-gastric influences (regurgitation, psychic secretion). These are considerations which have not received adequate attention in many of the human studies.

Although the results are not consistent and clear-cut in human experimentation, they are completely so in animal experimentation. It has been shown by several observers (6, 3, 4) that the secretory response of the Pavlov pouch dog to a meal can be abolished by atropine. Uschakov (7) found atropine effective against the reflex or cephalic phase of secretion whether induced by vagus stimulation or sham-feeding; that the gastric phase is also abolished was shown by a number of observers (8, 9, 10); Ivy, Lim, and McCarthy (11) have reported the same for the intestinal phase, a fact confirmed in the present work. It has been reported that atropine does not abolish the action of parenterally administered secretagogues. However, Kim and Ivy (12), who are the only ones who have employed secretagogues (liver extract and Liebeg's extract) *from which all histamine had been removed*, have reported that atropine is equally effective when the secretagogue is applied to the stomach, to the intestine, or when given parenterally. Klein (4) using mucosal transplants demonstrated that atropine can abolish a purely humoral secretion, presumably mediated by the hormone gastrin or by secretagogues. Yet atropine is unable to abolish histamine secretion even when the latter is no greater in rate than that which occurs during the intestinal phase which in point of volume and rate of secretion is the least important of the three phases. One must conclude that either histamine is not gastrin, or atropine prevents its formation.

CONCLUSIONS

1. The inhibitory effect of atropine on gastric secretion in total pouch dogs was determined quantitatively by measuring the extent to which it reduced a constant rate of gastric secretion produced by regularly repeated injections of histamine.

2. It was found that atropine produced a definite but limited inhibition which increased with the dosage. The inhibition produced by a given dose of atropine was less the greater the histamine stimulus.

3. Atropine occasionally increased the acidity of the gastric juice even though the rate of production of acid was markedly reduced.

4. It was shown that although atropine can completely abolish the intestinal phase of gastric secretion, it can only partially depress a quantitatively similar rate of secretion produced by repeated histamine injections.

The significance of this fact for the gastrin theory is discussed, it being concluded that either histamine is not the humoral gastric secretory excitant, or atropine prevents its formation when a meal is ingested.

REFERENCES

(1) Sacks, Ivy, Burgess and Vandolah. This Journal **101:** 331, 1932.
(2) Gray, Bradley and Ivy. This Journal **118:** 463, 1937.
(3) Keeton, Luckhardt and Koch. This Journal **51:** 469, 1920.
(4) Klein. Arch. Surg. **26:** 246, 1933.
(5) Polland. J. Clin. Investigation **9:** 319, 1930–31.
(6) Riegel. Ztschr. f. klin. Med. **37:** 381, 1899.
(7) Uschakov. Dissertation St. Petersburg. 1896. Quoted by Babkin.
(8) Sanozki. Dissertation St. Petersburg. 1893. Quoted by Babkin.
(9) Zeliony and Sawitch. Pflüger's Arch. **150:** 136, 1913.
(10) Lim, Ivy and McCarthy. Quart. J. Exper. Physiol. **15:** 13, 1925.
(11) Ivy, Lim and McCarthy. Quart. J. Exper. Physiol. **15:** 55, 1925.
(12) Kim and Ivy. This Journal **105:** 220, 1933.
(13) Altshuler. Arch. Int. Med. **42:** 117, 1928.

THE ORIGIN OF THE R AND T POTENTIALS FROM THE MAMMALIAN HEART[1]

J. A. E. EYSTER, M. R. KRASNO, C. A. MAASKE AND M. J. ULEVICH

From the Department of Physiology, University of Wisconsin

Received for publication June 7, 1937

When potential time curves are recorded from points at a known distance and direction from electrical charges in the center of a circular conducting field, the disposition of the charges responsible for the potentials present in the field can be determined from the magnitude, form and time relations of the recorded curves. The disposition of electrical charges in the tortoise and frog's heart (1) (2) and in the gastrocnemius muscle of the frog (3), as determined by the application of this principle, have been reported from this laboratory. Reference may be made to these reports for detailed description of the procedures employed. The present report is concerned with the same problem in the mammalian heart.

METHODS. The experimental procedures were identical with those previously reported except that the hearts were under artificial perfusion. Cats' and rabbits' hearts were placed at the center of a conducting field of saline solution, 43 cm. in radius and 5 mm. thick. Perfusion was through the aorta with oxygenated Locke's solution or defibrinated blood mixed with Locke's solution under a pressure of 60 mm. of mercury and at a temperature of 36°C. The outflow from the coronary system was into the surrounding saline, the level of which was maintained constant. Potential time curves were recorded, as in the previous work, at a constant distance from the heart along the various axes, fifteen degrees apart. An electrogram was recorded from leads on the base and apex of the ventricles, simultaneously with the field potential curves. (Fig. 1.)

RESULTS. As in the previous work on the tortoise and frog heart, it was found that four asynchronous dipoles within the heart would account, to a high degree of approximation, for the potential distribution in the surrounding field. Two of these dipoles are responsible for the R potentials, two for the T potentials. A greater degree of difference between different hearts in reference to the exact orientation of these dipoles was found than in the experiments on the tortoise and frog. This variability is probably due to the greater viability of the mammalian heart when isolated and the experimental difficulties of maintaining it under conditions approximating those of the heart in situ. Experiments were

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

included in this study only when the records obtained from the zero and the hundred eighty degree axes were mirror images of each other and where a final record checked closely with one obtained on the same axis early in

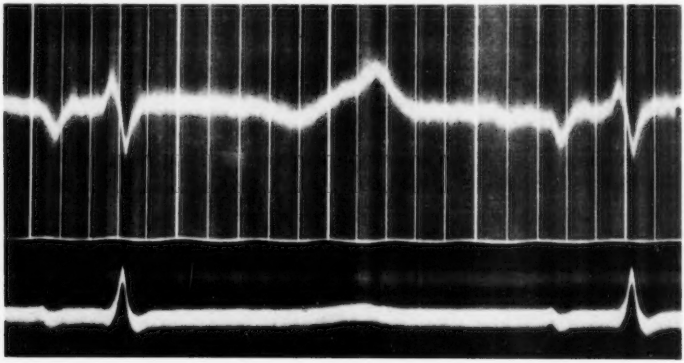


Fig. 1. The upper curve is a record of potential changes in a circular conducting field surrounding an isolated cat's heart at a point along an axis 15° from the cross axis of the heart and 14.25 cm. distant from the middle of the ventricles. The vertical lines mark time intervals of 0.04 sec. The sensitivity of the recording apparatus was one microvolt per millimeter. An upstroke on the curve indicates a fall of potential. The lower curve is an electrogram from leads applied to the heart.

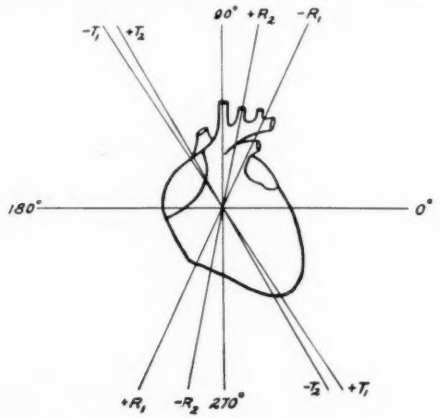


Fig. 2. Average orientation of the dipoles associated with ventricular potentials. The two labeled R are associated with the R group, the two labeled T with the T group of potentials. The subscripts denote the order of occurrence in each group.

the series of observations. This criterion gave the assurance that there had been no important change in the orientation of charges or in the type of heart beat during the course of the experiment. In 9 experiments this constancy was maintained throughout the period (approximately an hour)

necessary to obtain the field potential records. The greatest degree of correspondence as regards dipole orientation in different hearts occurred in four of these in which the perfusion solution contained a large proportion of defibrinated blood.

The average orientation[2] of the four dipoles is indicated in figure 2. The first electrical change in the ventricles appears as negative charges in the region of the left ventricular base and positive charges to the right of apex (R_1 dipole). This is followed in a short time by a reversal of charge orientation and a shift of the dipole axis to the right (R_2 dipole). The electrical charges in the heart associated with the T potentials are oriented in general between the right ventricular base and apex. During the inscription of the T wave the base is at first negative (T_1 dipole) and then positive (T_2 dipole) with respect to the apex.

In a considerable number of the additional experiments performed, in which the records obtained did not meet the criterion described above, the heart appeared to beat normally throughout the period of observation. The ventricles contracted vigorously and showed no evident areas of asystole. The shift in position of charge orientation indicates, however, that some changes of this nature were occurring, although not evident to visual observation. The isolated artificially perfused heart is at best a dying heart and relatively small imbalances of activity between different parts would be expected to develop after a relatively short period and result in alterations of potential distribution.

In several instances the records revealed an electrical alternans where there had been no evidence of mechanical alternans. The electrical alternans consisted in a different orientation of the T dipoles, the R dipoles maintaining the same direction in the two types of activity. The shape, size, duration and time of onset of the T complexes were often markedly different.

SUMMARY

The disposition of electrical charges in the isolated mammalian heart responsible for the ventricular R and T potentials is determined by analysis of potential-time curves recorded from a circular conducting field surrounding the heart. As in the previous work on the tortoise and frog heart, it is found that two groups of two associated positive and negative charges or dipoles, developing asynchronously, will account, to a high degree of approximation, for the field potentials observed.

REFERENCES

(1) Eyster, J. A. E., F. Maresh and M. R. Krasno. This Journal **110:** 422, 1934.
(2) Krasno, M. R., J. A. E. Eyster and C. A. Maaske. This Journal **114:** 119, 1935.
(3) Eyster, J. A. E., F. Maresh and M. R. Krasno. This Journal **111:** 641, 1935.

[2] The method by which the direction of the dipole axes is determined from the potential time curves recorded from the field around the heart has been described in detail in a previous publication (2).

THE ELECTRICAL POTENTIALS OF THE COCHLEA AND AUDITORY NERVE IN RELATION TO HEARING[1]

JOHN GUTTMAN AND S. E. BARRERA

From the Department of Neuropathology, New York State Psychiatric Institute and Hospital, New York, N. Y.

Received for publication June 7, 1937

In previous publications concerning the so-called Wever and Bray phenomenon, the authors (1a, b) showed that the cochlear electrical response could be elicited one to six weeks after intradural section of the eighth nerve, despite the fact that a degeneration of the cochlear ganglia and auditory nerve was demonstrable histologically. To meet possible criticism to the effect that six weeks was too brief a post-operative period to produce a change in the cochlear electrical response in cats, the authors have examined the response in six cats in which similar eighth nerve section was performed two to eighteen months before the electrical test and in which destruction of the opposite inner ear was performed at the time of the test. It was found not only that the cochlear response was present but also that in some cats, which were previously conditioned for audition and vision, the hearing sensation was destroyed by the nerve section.

PROCEDURE AND APPARATUS. Cats were used in all the experiments. Under ether anesthesia the cerebellum was exposed by a posterior occipital approach and the dura opened. The lateral lobe of the cerebellum was retracted medially, exposing the eighth and seventh nerves, which were cut by encircling the nerve mass with a small hook knife and drawing the knife dorsally. When the nerves were cut there was usually a sharp twitch of the muscles of the same side of the face. Following operation a paralysis of the seventh nerve was found with inability of the animal to close the eye.

The apparatus used for the electrical tests was a modified form of that used by one of us (G) (2) in earlier experiments. The apparatus producing the sound consisted of an audio-oscillator from which the sound frequencies, after passing through an attenuator, amplifier and loud speaker, were led through a rubber tube into the external auditory canal of the ear investigated. The apparatus for indicating the cochlear response

[1] This research was assisted by a grant from the Committee on Scientific Research of the American Medical Asociation.

included an input circuit of an amplifier, the low side of which was grounded. The output circuit of the amplifier was led into ear phones through which the observer could easily pick up the electrical response of the cochlea.

OBSERVATIONS. The results found in a typically conditioned animal may be represented by the following case:

Cat 3 was conditioned for light and sound for three months after which it responded properly to the given stimuli. The right eighth nerve was then sectioned and four weeks after operation reconditioning was again initiated. The cat responded promptly to the stimuli. Eight months later the left cochlea was destroyed by inserting a needle into the round window and producing an injury in the scallae of the cochlea. This destroyed the cochlear response. Testing thereafter showed that the cat was deaf, not responding to auditory stimuli but responding to light stimuli.

The effects of sound entering the normal ear of an animal may be summarized in terms of three effects: the cochlear electrical potential, the auditory nerve electrical potential and the sensation of hearing.

1. *Cochlear electrical potential.* It seemed advisable to check, espepecially in the light of conflicting reports, factors interfering with the cochlear response and factors not influencing it. In our experiments it was found that the cochlear electrical potential is destroyed by: *a*, artificially producing bleeding in any of the scallae; *b*, chipping off part of the rim of the round window; *c*, destruction of the bony septum between the middle ear and cochlea; *d*, injection into the round window of some drugs such as nicotine, quinine, etc. We found that the cochlear response was not destroyed by such factors as: *a*, perforation of the secondary tympanic membrane; *b*, withdrawal of a minute amount of perilymph by a hypodermic syringe and small needle; *c*, section of the eighth nerve.

The origin of the cochlear response has been and still is the subject of much controversy. Originally it was thought that the cochlear electrical disturbance was of neurogenic nature, being merely action currents transmitted from the eighth nerve (Wever and Bray, 3). Later it was considered to be of mechanical, microphonic origin (Adrian, 4; Kreezer, 5). At present, some investigators compromise by considering the response to be 75 per cent of cochlear origin with an admixture of 25 per cent of nerve action potential (Davis, 6). The neurogenic origin of the cochlear electrical potential is assumed by Hallpike and Rawdon-Smith (7), who found that the cochlear electrical disturbance was absent six months after sectioning of the eighth nerve intracranially.

The report of Hallpike and Rawdon-Smith was of particular interest to us because the results of our own experiments, as previously reported, apparently were in direct contradiction to theirs. In our cases of section

of the eighth nerve intradurally, there was also a degeneration of the cochlear ganglia. However, in such cases, even six weeks after operation, we could detect no appreciable change in the cochlear electrical potential. The only possible explanation which we have been able to discern for the radical differences and results is based upon a question of operative technique. The operative methods in the two experiments differ radically. In our cases the section of the nerve was performed with visualization of the nerve trunk after the dura had been opened and the cerebellum carefully retracted from the lateral wall of the posterior fossa. As Hallpike and Rawdon-Smith themselves state, in their cases they did not open the dura but inserted a blunt probe through the dura blindly into the internal auditory meatus, crushing the nerve. We have repeated the operative procedure using, as we interpreted it, their technique. In most of the cases so investigated it was possible to immediately eliminate the response by this operative method. This has indicated to us the possibility that the cochlear response in their cases may possibly have been eliminated not merely by destruction of the nervous elements of the eighth nerve but by direct, mechanical injury to the bony structure of the cochlea produced by the probe being inserted into the internal auditory meatus at the time of operation. Inasmuch as we have been able to elicit the response on repeating our experiments and carrying the post-operative period up to eighteen months, it would seem to be indicated that the response can occur even after complete degeneration of the nervous elements.

Figures 1 and 2 are two microphotographs of the inner ears of two cats as chosen from serial sections of the temporal bone and stained by the H. and E. methods. Figure 1 is from a normal cat and illustrates the normal eighth nerve trunk and cochlear ganglion. Figure 2 is a section from a cat in which the eighth nerve had been sectioned eighteen months prior to sacrifice of the animal. The complete disappearance of the eighth nerve fibers and cochlear ganglion cells can be clearly seen.

In the present work, as in the previous work, the nerve was severed carefully. It would be extremely difficult in such operative procedures to determine exactly the vascular status following the operation. Direct or indirect traumatic section or thrombosis of the auditory artery would be extremely difficult to exactly determine. We share the opinion of Hallpike and Rawdon-Smith that the exact condition of the internal auditory artery at the time of the nerve section must indeed be determined largely by chance factors inevitably associated with an operative procedure of this type. Despite the probable operation of such chance factors, the cochlear response was obtainable in all of our animals indicating either that, as seems probable from our histological preparations, necrotic changes did not develop or that if they did, stages in their development occurred in which the cochlear response could be obtained.

2. *Auditory nerve potential.* We exposed the right cochlea and, leading off from the round window, we obtained an electrical potential. We then exposed the right auditory nerve and obtained an electrical potential from the nerve. We then destroyed the right cochlea through the round window and following this the nerve potential disappeared.

The auditory nerve potential is probably similar to that of any other sensory nerve except for the fact that it depends upon the integrity of the cochlear electrical potential since, as we have found, it was destroyed simultaneously with the abolition of the cochlear electrical potential.

Fig. 1

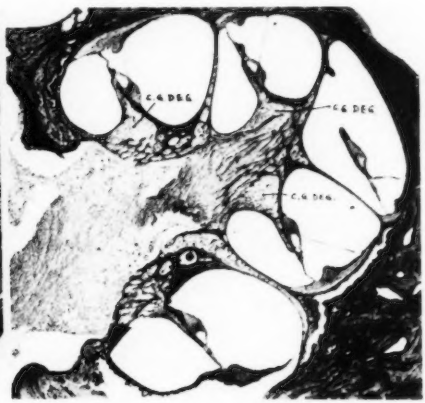


Fig. 2

Fig. 1. Section of the cochlea of a normal cat. The cochlear ganglion (*C. G.*) in its various portions can be clearly seen to be full of normal nerve cells.

Fig. 2. Section of the cochlea from a similar region as that of figure 1 but from a cat in which the ipsilateral eighth nerve had been sectioned intracranially eighteen months previous to sacrifice of the animal. The degenerated cochlear ganglion in its various portions (*C. G. Deg.*) can be clearly seen. The cochlear response was obtained from this cochlea.

3. *Hearing sensation.* It can be stated that hearing depends upon the basic integrity of the auditory nerve. In the earlier investigations on the nature of the cochlear electrical potential, it seemed possible that the electrical cochlear potential was a true action potential accompanying the auditory nerve impulse. It was not clear then how the electrical potential in the cochlea could persist without the nerve impulse which was absent after section of the auditory nerve and subsequent degeneration of the cochlear ganglia and auditory nerve endings. It seemed then that the cochlear electrical potential was associated either with the nerve impulse from some other nerve, the possibility of which was indicated by the finding of Bast (8) that the cochlear response could be obtained when one

electrode was placed on the trunk of the tenth nerve, or that the electrical potential from the cochlea is not a true action potential and is not associated with any nerve impulse. In light of the results to date, however, it would appear that hearing depends upon the integrity of the auditory nerve potential and this again depends upon the integrity of the cochlear electrical potential. A cochlear electrical potential is possible without an auditory nerve potential since the cochlear potential persisted even after section and degeneration of the eighth nerve. However, a nerve action current associated with hearing does not seem to be possible without a cochlear electrical potential since, as we stated above, the nerve electrical potential disappeared after destruction of the cochlear potential.[2]

SUMMARY

1. The eighth nerve was cut in six cats subdurally. The cochlear electrical response still persisted when the animals were tested two to eighteen months post-operatively. Subsequent histological studies demonstrated degeneration of the cochlear ganglion and nerve.

2. Hearing, as indicated by positive responses in conditioned animals, seems to be dependent upon the integrity of the eighth nerve and is accompanied by the action current of the eighth nerve. The action current of the eighth nerve is in turn dependent upon the integrity of the cochlear potential. The cochlear potential, however, does not depend upon the action current of the eighth nerve.

REFERENCES

(1a) GUTTMAN, J. Laryngoscope (Dec.) 1933.
(1b) GUTTMAN, J. AND S. E. BARRERA. This Journal **109:** 704, 1934.
(2) GUTTMAN, J. Laryngoscope (Dec.) 1933.
(3) WEVER, E. G. AND C. W. BRAY. J. Exper. Psychol. **19:** 143, 1936; also J. Comp. Psychol. **22:** 361, 1936.
(4) ADRIAN, E. D. This Journal **71:** 28, 1931.
(5) KREEZER, G. Am. J. Psychol. **44:** 638, 1932.
(6) DERBYSHIRE, A. J. AND H. DAVIS. This Journal **113:** 477, 1935.
(7) HALLPIKE, C. S. AND A. F. R. SMITH. This Journal **83:** 243, 1934.
(8) BAST, T. H., R. NOER, R. WEST, O. L. BACKUS, M. KRASNO AND J. A. E. EYSTER. Proc. Society for Exper. Biol. and Med. **30:** 638, 1933.

[2] We wish to acknowledge with thanks the services of Dr. Charles N. Winslow in conditioning our cats to sound and light, and of Mr. Martin Posner in devising and operating the electrical apparatus.

THE RELATION OF AMNIOTIN TO THE BASAL METABOLISM OF THE THYROIDECTOMIZED RAT

T. C. SHERWOOD, T. M. WILSON AND H. BONETA

From the Department of Anatomy and Physiology, University of Kentucky, Lexington

Received for publication June 10, 1937

A series of studies published during the last four years indicates that the administration of certain glandular extracts reduce the basal metabolic rate.

VanHorn (1933) reported a possible quantitative relationship between the secretion of the thyroid and follicular hormones. Sherwood, Savage and Hall (1933) found a definite decrease in the basal metabolism of adult ovariectomized rats given amniotin. Starr and Patton (1934) observed a decrease in the basal metabolism of hyperthyroid individuals given theelin and antuitrin S.

Sherwood and Bowers (1936) reported a decrease in the basal metabolic rate of adult ovariectomized rats when amniotin was injected. In the same study it was found that experimental hyperthyroid animals returned to a normal basal metabolic rate in approximately one-half the usual time when amniotin[1] was administered.

Several months later Gessler (1936) reported that folliculin diminished the abnormally high basal metabolism of guinea pigs that had received thyroid extract in spite of the simultaneous administration of quantities of thyroid extract, which, given alone, would have been sufficient to maintain the basal metabolism at its high level. Normal guinea pigs also showed a diminished basal metabolic rate after the injection of folliculin.

In view of these findings it seems desirable to determine the mechanism responsible for the effects noted. The present investigation was therefore designed to study the effect of amniotin upon the thyroidectomized rat.

EXPERIMENTAL. Eighteen adult ovariectomized rats were thyroidectomized under the binocular microscope, thus insuring a careful removal of the gland with no injury to the laryngeal nerves.

The animals were maintained in a room of thermic neutrality (28°C.) both summer and winter thus eliminating a possible change in the basal metabolic level due to temperature variations.

The basal metabolic rate was determined at 28°C. and the data calcu-

[1] The amniotin used in the present investigation was generously supplied by E. R. Squibb and Sons.

lated in calories per kilogram of body weight and per square meter of body surface.

The animals were fasted twenty hours prior to each test which was taken between the hours 10 a.m. and 4 p.m. This period of the day was used since very little activity was observed in the animals.

The normal metabolic rate was determined prior to thyroidectomy. The ten-day postoperative period gave the animals sufficient time for complete recovery. Following this period, another series of tests was made for the purpose of observing the hypothyroid basal metabolic level.

TABLE 1

The basal metabolism of the rat

RAT NUMBER	AVERAGE WEIGHT	CALORIES PER SQ./M./HOUR			AVERAGE WEIGHT	CALORIES PER SQ./M./HOUR		
		Diack*	Lee*	Rubner*		Diack	Lee	Rubner
		Controls				Thyroidectomized		
	grams				*grams*			
1	228	38.1	32.8	31.0	233	25.1	23.8	22.6
2	216	38.5	33.1	31.6	212	31.3	26.8	25.9
3	232	42.3	36.4	34.7	240	31.0	26.6	25.5
4	191	41.1	35.2	34.0	203	32.0	27.4	26.3
5	212	40.6	34.8	33.3	222	26.8	23.4	22.4
6	220	38.1	32.8	31.2	228	28.3	24.4	23.2
7	217	38.4	33.6	32.1	218	29.5	25.4	24.2
8	199	39.7	34.0	32.6	215	33.6	28.9	27.8
9	214	40.2	34.5	33.1	219	29.5	25.4	24.2
10					210	28.5	24.3	23.3
11	197	39.4	33.7	32.3	210	30.1	25.8	24.8
12	218	37.8	32.9	31.4	222	28.0	23.5	22.4
13	204	39.6	33.9	32.2	213	33.6	28.9	27.3
14	195	40.9	34.8	33.3	199	30.6	26.2	25.1
15	169	41.5	35.1	33.7	202	31.4	26.9	25.9
16	186	38.4	32.6	31.4	203	30.0	25.7	24.6
17					229	31.5	27.1	25.8
18	217	39.7	34.3	32.7	220	28.2	24.3	23.2

* Diack—$S = 7.47\ (W)^{\frac{2}{3}}$. Lee—$S = 12.44\ (W)^{\frac{1}{2}}$. Rubner—$S = 9.1\ (W)^{\frac{1}{3}}$.

When the hypothyroid level had been established, each animal was injected with one thousand international units of (Squibb) amniotin daily for a period of three days. The basal metabolism tests were continued for several weeks following the amniotin therapy.

RESULTS. The thyroidectomized rats gave no indication of being abnormal insofar as weight and general health were concerned.

Table 1 presents the average weight and the basal metabolic rate as calculated by the surface area formulae of Diack (1930), Lee (1929) and Rubner (1883). These averages are presented for the control period

before thyroidectomy as well as for the period following thyroidectomy. Animals 10 and 17 were not used in the control series. All other animals showed a decrease in the basal metabolic rate following thyroidectomy.

Table 2 presents the average weight, the basal heat production following thyroidectomy as calculated by the surface area formula of Lee (1929), the daily amniotin dosage given to the hypothyroid animals and the basal heat production following amniotin therapy. The data presented in table 2 give no indication of a further decrease in the basal metabolic rate following the administration of amniotin to experimental hypothyroid rats.

TABLE 2

Amniotin-metabolism relationship in the thyroidectomized rat

RAT NUMBER	AVERAGE WEIGHT	CALORIES PER SQ./M./HOUR LEE* FOLLOWING THYROIDECTOMY	DAYS THERAPY	UNITS AMNIOTIN DAILY	CALORIES PER SQ./M./HOUR LEE* FOLLOWING AMNIOTIN THERAPY
	grams				
1	233	23.8	3	1,000	24.2
2	212	26.8	3	1,000	25.1
3	240	26.6	3	1,000	26.0
4	203	27.4	3	1,000	27.0
5	222	23.4	3	1,000	25.0
6	228	24.4	3	1,000	24.8
7	218	25.4	3	1,000	24.8
8	215	28.9	3	1,000	27.2
9	219	25.4	3	1,000	26.3
10	210	24.3	3	1,000	25.0
11	210	25.8	3	1,000	24.7
12	212	23.5	3	1,000	22.8
13	213	28.9	3	1,000	27.6
14	199	26.2	3	1,000	26.8
15	202	26.9	3	1,000	26.0
16	203	25.7	3	1,000	24.9
17	229	27.1	3	1,000	26.3
18	220	24.3	3	1,000	24.0

* Lee's formula was used in the determination of the basal metabolic rate.

Discussion. Since it has been shown by Sherwood and Bowers (1936) and Gessler (1936) that the basal heat production is decreased in hyperthyroid animals given ovarian hormones, an attempt was made to show the relation of the thyroid to the mechanism responsible for the decreased heat production when amniotin is administered, by observing the effect of amniotin upon the experimental hypothyroid rat.

As has been stated in a previous report of the senior author (1933) the hypophysis may have been responsible for the decrease in heat production

when amniotin had been administered. However, the removal of the thyroid gland brought about a decrease in the basal heat production which was approximately the decrease noted when amniotin injections were given to the otherwise normal ovariectomized animal.

That the thyroid does have an effect upon the ovarian function has been shown by VanHorn (1933). Three rat units of theelin were necessary to produce oestrus in the experimental hyperthyroid rat where one rat unit was sufficient in the normal animal.

Recently collected data of this laboratory indicate that the hormones of the thyroid and ovary are in some way related to each other in the control of basal metabolism. Further investigation is necessary to show whether or not the presence of the thyroid gland is necessary for this relationship.

CONCLUSIONS

Amniotin has no measurable effect upon the basal metabolism of the thyroidectomized rat.

We have pleasure in expressing our thanks to E. R. Squibb and Sons for their amniotin; to Doctor and Mrs. M. M. White for their helpful criticism of the manuscript.

REFERENCES

DIACK, S. L. J. Nutrition **3:** 289, 1930.
GESSLER, C. Arch. Inter. de Pharm. et de Therap. **54:** 263, 1936.
LEE, M. O. This Journal. **89:** 24, 1929.
RUBNER, M. Ztschr. f. Biol. **19:** 536, 1883.
SHERWOOD, T. C., M. SAVAGE AND J. F. HALL. This Journal **105:** 241, 1933.
SHERWOOD, T. C. AND L. M. BOWERS. This Journal **115:** 645, 1936.
STARR, P. AND H. PATTON. Endocrinol. **18:** 113, 1934.
VANHORN, W. M. Endocrinol. **17:** 152, 1933.

LOSS OF POTASSIUM IN VOLUNTARY CONTRACTION

WALLACE O. FENN

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication June 12, 1937

There is now ample evidence (Fenn and Cobb, 1935) that in electrically stimulated muscles of cats, rats, and frogs there is a loss of potassium in exchange for sodium and a gain in water. There is, however, no evidence that the same change occurs during voluntary contraction. Proof of such a change in man must depend upon analyses for potassium in the blood. Dill, Talbot and Edwards (1930) have shown a loss of water from the blood during exercise but no change in potassium. Other pertinent reports in the literature are conflicting and have recently been reviewed (Fenn, 1936). Even in the blood of cats during electrical stimulation sufficient to make the muscles lose considerable amounts of potassium, the change of plasma potassium is minimal. Thus, any potassium that escapes into the blood is presumably taken up somewhere else in the body, and in order to prove a loss of potassium in voluntary activity, it becomes necessary to analyze the muscles themselves. This we have attempted to do in a series of eighteen rats.

METHOD. One sciatic nerve was cut with aseptic precautions under ether anesthesia. One to seven days after the operation, the animals were thrown into a water bath where they were obliged to swim until they were exhausted. They were then killed by a blow on the head and their throats cut to drain as much blood from the muscles as possible. The muscles were then dissected, put into tared weighing bottles, and water contents determined after drying to constant weight at 100°C. The dried samples were transferred to platinum crucibles and ashed in an electric furnace for 15 hours at 500°C. and then analyzed in duplicate for potassium by methods previously described (Fenn and Cobb, 1935). These two analyses were averaged for the final result. The average difference between duplicate analyses of the same sample of muscle ash was 1.3 per cent and was seldom over 3 per cent. Due to errors in sampling the fresh muscles, two samples of the same muscle, weighed, ashed, and analyzed separately may differ by as much as 5 per cent. A control series of rats was treated the same way except that they were not exercised in the water bath before killing.

The results of these analyses are included in table 1 where data from both the control and exercised series are shown. Four different muscles were analyzed from each hind leg of the rat. Nine rats were used in each series, the time after operation varying from one to seven days. The actual contents of denervated muscles are listed in the table as well as the differences between the denervated and the innervated muscles. A plus sign indicates more potassium or water in the normally innervated muscle.

TABLE 1

Analytical results

Potassium and water are expressed in milli-equivalents and cubic centimeters per 100 grams dry weight, respectively. The actual contents in potassium and water of denervated muscles are given as well as the differences found between the innervated and denervated muscles. A plus sign indicates more potassium or water in the innervated muscle.

DAYS	GASTROCNEMIUS				TIBIALIS				BICEPS FEMORIS				SEMIMEMBRANOSUS			
	K	H_2O	ΔK	ΔH_2O	K	H_2O	ΔK	ΔH_2O	K	H_2O	ΔK	ΔH_2O	K	H_2O	ΔK	ΔH_2O
Control rats																
1	46.6	316	−2.5	+5	48.0	316	−3.8	−5	46.5	324	+0.1	+1	48.4	325	−0.1	−4
1	47.3	329	−1.8	−5	50.0	330	−4.8	−11	49.0	349	−1.6	−16	50.8	343	−2.3	−7
2	46.1	329	−1.8	−3	47.4	318	−3.2	−4	47.7	332	−2.9	−9	48.6	336	−2.2	−9
3	47.2	335	−2.2	−7	45.1	322	+0.5		47.5	328	−4.2	+6	48.0	340	+0.1	+6
4	50.1	327	−6.0	−7	46.9	335		−20		336		−8	47.5	341	+1.2	−17
4	50.0	347	−4.6	−5	45.1	334	+0.7	−14	47.2	378	−1.0	−12	50.6	358	+0.4	−12
7	52.1	347	−3.0	−21	47.1	338	+0.1	−26	47.9	340	+1.8	−8	51.7	351	+0.3	−27
7	47.5	345	+1.8	−19	50.0	319	−0.4	−7	48.4	353	+1.6	−23	50.9	374	+0.4	−48
7	44.7	350	−1.0	−19	41.6	339	+4.5	−21	41.2	356	+6.3	−22	47.0	360	+2.0	−14
Exercised rats																
1	49.5	328	−4.5	−2	47.9	319	−2.7	−3	47.5	329	−5.4	−5	49.2	363	−3.2	−23
1	48.9	322	−4.5	+30	49.8	316	−3.9	+11	47.0	331	−2.3	+54	50.9	333	−5.8	+49
2	47.2	329	−3.1	+55	44.9	313	−1.9	+37	47.4	335	−5.3	+62	49.5	348	−3.9	+73
3	49.2	332	−4.2	+28	48.6	318	−1.6	+18	47.5	329	−1.4	+66	52.3	332	−3.3	+46
4	43.5	312	−1.3	+28	45.9	314	−3.2	+23	43.7	322	−2.2	+41	43.7	322	−2.2	+41
4	48.4	330	−4.4	+2	48.6	337	−2.6	−19	48.1	356	−5.4	+30	53.0	354		+15
7	48.8	346	−1.1	+50	43.1	352	+1.9	+31	48.0	364	+1.6	+51	52.5	365	−2.3	+51
7	52.0	341	−6.7	+34	46.1	328	+0.2	+12	49.4	343	−3.3	+70	53.0	362	−5.9	+53
7	44.6	345	−0.8	+29	43.1	340	+0.5	+7	41.9	348	−2.1	+51	46.0	369	−6.2	+29

The significant points brought out by these data are best seen in table 2 where the average changes in potassium and water content for the different muscles of the two series are tabulated.[1] In the control series it is seen that the denervated muscle contained uniformly more water and slightly more potassium than the innervated muscle. The increased water may be due to an increased filtration of water from the capillaries as a result of

[1] This same table in a preliminary form was published in the Cold Spring Harbor Symposium **4**: 252, 1936.

a vasodilatation following the denervation. The gain in potassium after denervation may possibly be attributed to the complete rest which the muscle enjoyed.[2] The gain in potassium by the denervated gastrocnemius is much greater than that observed in the other three muscles, presumably indicating that this antigravity muscle is particularly active in cage life. In the exercised series, all the innervated muscles gained water and lost potassium. The loss of potassium was greater in every case than the loss on the control side, although the difference is not certainly significant in every case (cf. gastrocnemius and tibialis). This may be taken to mean that, for the muscles concerned, the exercise of swimming is proportionately greater than the exercise involved in walking around the cage. Apparently, by this technique, it is only possible to compare two different degrees of exercise; it is not possible to obtain for analysis a completely resting and still normally innervated muscle. The interpreta-

TABLE 2

Average change in potassium and water compared to denervated side

	CONTROL		EXERCISED	
	K	H_2O	K	H_2O
	m.-eq.	*cc.*	*m.-eq.*	*cc.*
Gastrocnemius	−2.3	−9	−3.4	+28
Tibialis	−0.8	−12	−1.5	+13
Biceps femoris	−0.1	−10	−2.9	+47
Semimembranosus	−0.2	−15	−4.1	+37

A minus sign indicates less water or potassium in the normal or exercised muscle than in the corresponding denervated muscle. Quantities are in milli-equivalents of potassium or cubic centimeters of water per 100 grams of dry muscle.

tion of these data can only be improved by knowing quantitatively the amount of exercise involved in each muscle in the two series. Comparison of the figures in table 1 will show that the results were not entirely uniform in different rats. The variations are particularly marked in the potassium differences in the control series where some rats showed gains and some losses, most of them being small, however, and probably accounted for by the errors of the method. Other causes of variation in the exercised series will be discussed later.

It is instructive to average together the analyses obtained from all four muscles of each rat. The results are shown in table 3. The figures in the control series show the changes resulting from denervation at various times after operation. Some similar figures recalculated from Hines and

[2] The difference in potassium may also be accounted for in part by an excessive activity of the one normal leg after the other has been denervated.

Knowlton (1933) are included for comparison. For the sake of uniformity, the signs refer to the normally innervated muscles so that a minus sign indicates a loss by the innervated or a gain by the denervated muscle. The figures show that after denervation the muscles usually gain water for reasons already suggested. This is true during the first week, as well as after longer times when atrophy is pronounced, as shown by Hines and Knowlton. The potassium figures show, however, that during the first week the denervated muscles gain potassium, possibly due to lack of exercise, while after this time there is uniformly a loss of potassium corresponding to the atrophy. This initial gain in potassium was overlooked by Hines and Knowlton, who had only one figure during the first week. This one figure, obtained three days after denervation, showed a small loss of potassium instead of the uniform gain which is shown by the first seven of the rats in our series.

The figures from the exercised series in table 3 make possible certain suggestive correlations between the duration of the swim (last column) or other features of the exercise period and the chemical changes observed. Rats 1(a) and 4(b) (numbers used to indicate also the number of days since operation) gained the least water on exercise, rat 1(a) showing actually a loss of water in all four exercised muscles. Unlike the other animals, neither of these rats was exhausted when killed, and they swam the longest of any of the rats with the exception of 7(c), which gained the next smallest amount of water. Thus, *a small gain of water seems to be correlated with a long swim or low fatigability.* An actual loss of water on exercise, as in all four muscles of rat 1(a) is surprising, but we have observed a similar loss of water from rat muscles stimulated electrically for a period of three hours when no water was supplied to the rat (Fenn and Cobb, 1936). In general, the rats which swam at 20°C. seemed to become exhausted more rapidly than those which swam at 30°C. and *the longer the exercise, the greater the loss of potassium.* Thus, if the rats are arranged in order of the duration of swim, it will be found that the losses of potassium are in the same order, with the exception of rats 3 and 7(c). In rat 3, the duration of the swim was listed as 15 minutes, but this was divided into two periods separated by an hour, so that the exercise period was in effect longer, which may explain the slightly greater loss of potassium in this rat. In rat 7(c) the loss of potassium is too low to fit into the sequence of the other rats. This may well be experimental error, or it may be due to an incipient atrophy and loss of potassium from the denervated muscle, since the control series shows some such effect after seven days. In general, however, there seems to be little difference between rats during the first seven days after operation so far as the control series is concerned, while the exercise series shows a fairly good correlation with the duration of exercise. The experimental error is so large, however, that in a small series of this sort the relations cannot be regarded as proved.

TABLE 3

Average changes in potassium and water in each rat

DAYS AFTER OPERATION OR RAT NUMBER	CONTROL SERIES		EXERCISED SERIES		DURATION OF SWIM
	H_2O	K	H_2O	K	
	cc.	*m.-eq.*	*cc.*	*m.-eq.*	*min.*
1(a)	−0.8	−1.6	−8	−4.0	120
1(b)	−9.8	−2.6	+36	−4.1	25
2	−6.2	−2.5	+57	−3.6	20
3	+1.2	−1.4	+39	−2.6	15
4(a)	−13.0	−2.4	+33	−2.2	20
4(b)	−10.8	−1.1	+7	−4.1	60
7(a)	−20.5	−0.2	+46	0	15
7(b)	−24.3	+0.9	+42	−3.9	22
7(c)	−19.0	+2.9	+29	−2.2	60
Recalculated from Hines and Knowlton (1933)					
3	0	+2.4			
7	−11.8	+3.0			
14	−16.4	+3.3			
21	−0.9	+4.4			
28	−32.7	+13.1			
42	−13.2				

Each figure is the difference in average contents of four pairs of muscles from one rat. All figures are calculated per 100 grams of dry muscle. A plus sign indicates more potassium or water on the innervated than on the denervated side.

Rats 1(a) and 4(b) were described as "not exhausted" when killed. Rats 2 and 3 swam first for 10 minutes at 20°C.; then 10 minutes and 5 minutes, respectively, at 30°C. an hour later. Rat 7(a) (250 gm.) and 4(a) were large ones; rat 7(a) was the smallest (101 gm.). All were males except 7(b) and 7(c). Left sciatic was cut in three rats; otherwise the right.

TABLE 4

Average potassium and water contents of normal muscles and of denervated muscles of the control and exercised series

MUSCLE	POTASSIUM			WATER		
	Normal	Denervated		Normal	Denervated	
		Control	Exercise		Control	Exercise
	m.-eq. per 100 grams dry			*cc. per 100 grams dry*		
Tibialis	46.0 ± 0.37	46.8	46.4	317 ± 1.1	326	328
Gastrocnemius	45.9 ± 0.45	48.0	48.0	329 ± 1.4	332	336
Biceps femoris	46.8 ± 1.4	46.9	46.7	335 ± 2.7	340	344
Semimembranosus	49.1 ± 0.57	49.3	50.0	335 ± 2.5	350	348

Averaged potassium and water contents of four different muscles from all nine rats are shown in table 4. Only normal (nonexercised and innerv-

ated) muscles and the denervated muscles of both control and exercised series are included. The table shows the close agreement between the average values obtained in the two series of denervated muscles, which were almost identical both for potassium and for water. The averages for the normal muscles are, of course, slightly different in absolute values, but the water contents of the different muscles are in the same order. The probable errors of the means show that at least the larger differences between different muscles are just significant. These differences are presumably due to varying amounts of connective tissue which were included in the samples. The figures for normal muscles suggest that there is more potassium in the muscles which contain the most water. This would be expected if osmotic pressures are equal in the different muscles, since potassium is the chief cation present.

The averages for normal muscles in table 4 include one experiment not listed in table 1, and hence the values are slightly different from those given in table 1. In this control experiment the rat was operated in the usual way, the sciatic on one side being exposed but *not* cut, the other side remaining intact. Subsequent analyses of the muscles showed no differences; the average of seven analyses for potassium was 47.9 m.-eq. per cent dry weight on both sides, the corresponding water contents being 334 cc. per cent dry weight on the intact side and 337 cc. on the operated side.

SUMMARY

Rats with one sciatic nerve cut were exercised by swimming. The muscles sampled immediately afterwards and analyzed showed that voluntary contractions produced an increase in water content and a decrease in potassium. In general the muscles of rats which swam the longest and were least quickly fatigued lost the most potassium but gained the least water. Muscles sampled a few days after denervation showed a slight gain in potassium, presumably because of the lack of activity.

I am much indebted to Miss Doris M. Cobb for assistance in these experiments.

REFERENCES

Dill, D. B., J. H. Talbot and H. T. Edwards. J. Physiol. **69**: 267, 1930.
Fenn, W. O. Physiol. Reviews **16**: 450, 1936.
Fenn, W. O. and D. M. Cobb. This Journal **115**: 345, 1936.
Hines, H. M. and G. C. Knowlton. This Journal **104**: 379, 1933.

THE NITROGEN-SPARING ACTION OF GLUCOSE IN NORMAL, PHLORHIZINIZED AND DEPANCREATIZED DOGS[1]

I. ARTHUR MIRSKY, JOSEPH D. HEIMAN AND S. SWADESH

From the Department of Metabolism and Endocrinology, Institute for Medical Research, The Jewish Hospital, Cincinnati, Ohio

Received for publication June 12, 1937

The evidence that carbohydrate has the power to spare endogenous protein metabolism is both voluminous and conclusive. Thus, it is known that glucose ingestion will inhibit the increased protein metabolism that occurs in fasting, febrile conditions, hyperthyroidism and in phlorhizinized animals. However, it is still controversial as to whether the increased protein metabolism of the depancreatized dog is similarly affected by carbohydrate administration.

In a brief review and comprehensive investigation of the metabolism of the depancreatized dog, Barker, Chambers and Dann (1) recently reported that in the early and intermediate stages of inanition, the depancreatized dog exhibits no cessation of ketosis and no nitrogen-sparing effect following the *ingestion* of glucose. Their observations are in accord with those of the majority of workers on this problem. The few instances where contrary findings have been reported are so adequately criticised by these investigators as to invalidate the significance of such reports.

During the past year we demonstrated that a disappearance of ketone bodies from the blood and urine of the depancreatized dog can be produced by the *intravenous* administration of glucose in amounts adequate to produce a marked increase in blood sugar concentration and a deposition of glycogen in the liver (2). Subsequent studies led us to conclude that this effect of large amounts of glucose is due to the cessation of ketone body formation in the liver in consequence of an inhibition of fat catabolism by that organ (antiketogenic or "fat-sparing") (3) (4). The latter we attributed to the formation of amounts of glycogen adequate for the nutritional requirements of the liver itself.

In his studies on the phlorhizinized dog, Wierzuchowski (5) observed a parallelism between the antiketogenic action of ingested glucose and its nitrogen-sparing effect. These and other observations suggest that both

[1] Aided by the David May Fund.

Presented before the American Physiological Society April, 1937. Memphis, Tenn.

phenomena may be related and that whenever antiketogenesis is produced, a concomitant sparing of protein also will occur. If that be the case, then the intravenous administration of large amounts of glucose should produce a nitrogen-sparing effect in the comlpetely depancreatized dog receiving no insulin.

METHODS. The classical procedure employed in studies on the protein metabolism of an intact organism consists in determining the rate of nitrogen elimination in the urine. We employed this procedure during the preliminary stages of the present study, but found that the diuresis consequent to the intravenous administration of large amounts of glucose (or of an isosmotic solution of sodium sulphate) produced changes in nitrogen excretion which we could not regard as metabolic in origin. We therefore set out to find some other criterion for gauging protein metabolism.

In their studies on nephrectomized dogs, Deuel, Wilson, and Milhorat (6) utilized the rate of increase in the non-protein-nitrogen of the blood as an index of protein metabolism. We, therefore, studied the efficacy of this procedure and found it to be reliable. Thus, we found that if the urine of an anesthetized dog was reinjected into the blood stream as fast as it was secreted, the rate of rise of the non-protein-nitrogen of the blood was the same, within experimental limits, as that in the blood of a bilaterally nephrectomized dog. This indicates that the determination of the non-protein-nitrogen content of the blood of the nephrectomized dog over various periods of time is just as adequate an index of protein metabolism as is the determination of nitrogen excretion in the urine. Hence this method was employed throughout the present study, and all our data refer to nephrectomized animals.

Three sets of animals were studied, normal, phlorhizinized, and depancreatized dogs, the groups which did not receive glucose being regarded as controls for those to which glucose was administered. The normal animals were fasted from 72 to 96 hours before the experiment. Phlorhizin diabetes was produced in the second set of animals by the daily subcutaneous administration of one gram of phlorhizin suspended in oil, for three to four days, during which time the animals were fasted. The depancreatized animals consisted of a group from which the pancreas was removed carefully and completely, 96 hours before the actual experiment and during which interval no food or insulin was administered. In order to ascertain the effect of glucose in an incomplete pancreatic diabetes, only a partial pancreatectomy was performed in two animals.

In all experiments, the animals were anesthetized with sodium amytal and a bilateral nephrectomy performed. The anesthesia was maintained throughout the experimental period. Immediately after the completion of this operation an arterial blood sample was drawn from an exposed

femoral artery for the determination of non-protein-nitrogen by a micro-Kjeldahl[2] method, sugar by the Somogyi modification of the Shaffer-Hartmann method, and total solids by drying a weighed quantity of blood to constant weight. Arterial blood samples were drawn again at two to four hourly intervals for twelve hours. In a number of instances, the experimental period was extended an additional twelve hours in order to establish the constancy of the rate of protein metabolism observed under varying conditions.

Where glucose was employed a 30 per cent solution was administered intravenously at the rate of 2 grams per kilogram of body weight per hour for the first two hours, one gram per kilogram of body weight per hour for the next six hours and one-half gram per kilogram of body weight per hour for the remainder of the experimental period.

During the early stages of these studies, all non-protein-nitrogen and sugar values of the blood were corrected for changes in the water content,

TABLE 1

Examples of constancy of rise in blood non-protein-nitrogen after bilateral nephrectomy

TYPE OF EXPERIMENT	TOTAL RISE PER 12 HOUR PERIODS AFTER NEPHRECTOMY		RISE PER HOUR AFTER NEPHRECTOMY	
	First 12 hour period	Second 12 hour period	First 12 hour period	Second 12 hour period
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Normal dog	38.0	44.3	3.17	3.69
Depancreatized dog	58.1	60.7	4.84	5.06
Phlorhizinized dog	48.8	49.1	4.07	4.09

but this was subsequently abandoned because of the negligible changes observed.

RESULTS. Some of our pertinent data are summarized in tables 1 and 2 and figure 1, only the four hour blood samples being recorded. The typical experiments summarized in table 1 are presented to demonstrate that a twelve hour period after bilateral nephrectomy is representative of the rate of nitrogen metabolism since no significant alteration in the rate occurs when the studies are extended over a longer period of time.

Our data reveal that over a twelve hour period the blood non-protein-nitrogen in the normal control group rises at the average rate of 3.03 mgm. per cent per hour. In the phlorhizinized and the completely depancreatized control groups the rates are 3.93 mgm. per cent per hour and 5.14 mgm. per cent per hour respectively. This demonstrates that an increase in nitrogen metabolism occurs in both completely depancreatized and

[2] Digestion by the Koch-McMeekin method and distillation by the Bock and Benedict method.

TABLE 2

The blood non-protein-nitrogen of nephrectomized normal, phlorhizinized and depancreatized dogs, with and without glucose administration

EXPERIMENTS	NUMBER OF EXPERIMENTS	BLOOD SUGAR							BLOOD NON-PROTEIN NITROGEN						
		0	4 hours		8 hours		12 hours		0	4 hours		8 hours		12 hours	
		Actual	Actual	Change	Actual	Change	Actual	Change	Actual	Actual	Change	Actual	Change	Actual	Change
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Normal controls	5	66	66	0	66	0	70	+4	29.7	40.4	+10.7	52.3	+22.6	66.1	+36.4
Depancreatized controls	5	271	351	+80	423	+152	466	+195	45.1	67.5	+22.4	86.8	+41.7	106.8	+61.7
Phlorhizinized controls	5	50	106	+56	97	+47	104	+54	45.6	59.2	+13.6	74.7	+29.1	92.8	+47.2
Glucose treated normals	3	69	319	+250	105	+36	56	−13	26.7	31.1	+4.4	35.2	+8.5	43.7	+16.9
Glucose treated totally depancreatized	5	295	1406	+1111	1570	+1275	1705	+1410	39.0	56.7	+17.7	72.2	+33.2	80.0	+41.0
Glucose treated phlorhizinized	3	63	1009	+946	587	+524	434	+371	43.7	49.7	+6.0	53.8	+10.1	58.6	+14.9
Glucose treated partially depancreatized	2	102	750	+648	717	+615	618	+516	24.2	33.1	+8.9	40.9	+16.7	49.1	+24.9

phlorhizinized animals, and that this increase persists even in the absence of the kidneys. The difference in the rates of nitrogen metabolism is clearly illustrated when the data are computed on the basis of per cent deviation from the normal control group (fig. 1). Such an analysis reveals that the average rate at the end of a twelve hour period is 70 per cent faster in the depancreatized group and 30 per cent faster in the phlorhizinized group than it is in the normal control group. The degree of augmentation in the rate of protein metabolism in the completely depan-

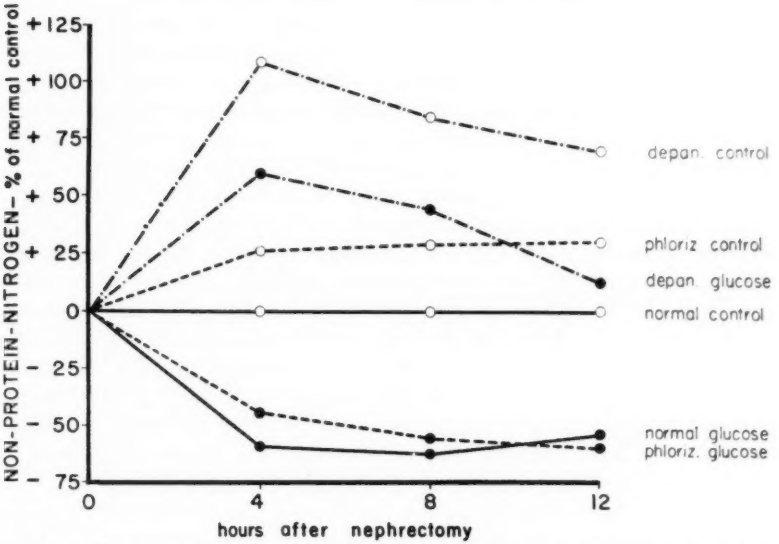


Fig. 1. Graphic comparison of the rates of protein metabolism as measured by non-protein-nitrogen accumulation in the blood of nephrectomized animals (table 2). The data are expressed as per cent of the normal control group. ———— normal; ——·——·— depancreatized; – – – – – – phlorhizinized; ○ without glucose injection; ● with glucose injection.

creatized animal is in accord with that observed by studying urinary nitrogen. However, in the phlorhizinized group our data indicate a lower rate of protein metabolism than is observed in phlorhizinized animals with intact kidneys.

The blood sugar changes in the three sets of the control experiments are not very significant, other than revealing that bilateral nephrectomy does not affect the blood sugar level of the normal animals; that it produces a linear increase in the blood sugar of the completely depancreatized animals, and a rise to the normal or slightly above the normal level in the phlorhizinized animals.

With the continued intravenous administration of glucose, the blood sugar of normal animals shows a relatively moderate increase with a subsequent return to the normal or even subnormal level. The administration of the same amount of glucose to the completely depancreatized animals results in a rapid accumulation of glucose in the blood to a concentration above 1400 mgm. per cent without the secondary decrease that is observed in the normal group. In the phlorhizinized animals a marked increase in the blood glucose concentration also occurs, but this is soon followed by a secondary decrease. The same response is noted in the partially depancreatized group as in the phlorhizinized animals.

The effect of glucose administration on the non-protein-nitrogen accumulation in the blood is very striking in all instances. In the normal animals, the rate of non-protein-nitrogen accumulation falls to 1.40 mgm. per cent per hour over a twelve hour period, and that of the completely depancreatized and phlorhizinized groups to 3.41 mgm. per cent per hour and 1.24 mgm. per cent per hour respectively. On basis of per cent change from the normal control group, we note that the intravenous administration of glucose results in a 53.6 per cent decrease in the rate of blood non-protein-nitrogen accumulation in the normal group, 89 per cent in the phlorhizinized, and 57.4 per cent in the completely depancreatized groups. However, the actual per cent of decrease for each group is 53.6 per cent in the normal, 33.5 per cent in the depancreatized, and 68.4 per cent in the phlorhizinized groups. The effect of glucose in the partially depancreatized group is intermediate between the response of the normal animals and that of completely depancreatized animals. These quantitative terms are employed merely to indicate the direction of the changes.

It is of interest to note that in both the normal and the phlorhizinized groups, a maximum decrease in the rate of nitrogen metabolism is obtained within four hours after the onset of glucose administration. However, in the completely depancreatized group, the effect of glucose administration is progressive so that a greater diminution in the rate is noted with each succeeding period. It is probable that in the latter group a more prolonged period of glucose administration would result in a still greater diminution.

Discussion. Our data reveal that a sparing of protein can be produced in the absence of insulin if glucose is administered intravenously in amounts adequate for glycogen deposition.[3] This is in accord with our previous observations and favors the possibility that the increase in protein metabolism after pancreatectomy and the decrease after the intravenous admin-

[3] It must be emphasized that in these studies we are dealing with animals in the early stages of inanition and not with severely undernutritioned animals where both Soskin (9) and Barker, Chambers and Dann observed glucose per os to decrease both nitrogen and fat metabolism.

istration of glucose may be due to a mechanism similar to that which we have postulated for ketogenesis and antiketogenesis (2) (3) (4). If that be so, then glucose may act by suppressing protein metabolism (i.e., oxidative deaminization) in the liver. Preliminary studies in this regard indicate that "nitrogen-sparing" occurs when the liver is removed from the diabetic dog.

The negative results of Barker, Chambers and Dann and of others may be attributed to the difficulty of producing a deposition of glycogen in the liver with orally administered glucose as compared to the ease with which this is accomplished when glucose is injected intravenously. It is obvious, also, that a lack of response to ingested glucose does not indicate an inability to oxidize carbohydrate as is indicated by the above and by other observations that the tissues of the diabetic dog can utilize carbohydrate even in the absence of insulin (11) (12) (13).

The data which we have discussed in this and previous reports are in accord with the hypothesis that the essential disturbance in pancreatic diabetes is an inability of the liver to retain an adequate amount of glycogen for its own utilization. In consequence of this, a compensatory acceleration of fat catabolism and oxidative deaminization then occurs in this organ and results in the gluconeogenesis which is known to take place in this condition. When an adequate concentration of glucose reaches the liver either as a result of the compensatory gluconeogenesis or from exogenous sources, glycogenesis exceeds glycogenolysis and a cessation of fat and protein catabolism in the liver ensues. This hypothesis is in accord with the recent views on substrate competition in the liver offered by Edson (14) and others in explanation of antiketogenesis.

SUMMARY AND CONCLUSIONS

1. The rate of accumulation of non-protein-nitrogen in the blood of nephrectomized animals can be used as a measure of the rate of protein metabolism.

2. The rate of nitrogen metabolism is greater than that of the normal in both phlorhizinized and completely depancreatized dogs even in the absence of the kidneys.

3. The intravenous injection of large amounts of glucose to anesthetized fasted, normal, phlorhizinized, and completely depancreatized dogs during the early stages of inanition, results in a decrease in the rate of protein metabolism in all instances.

4. These studies lend support to the hypothesis that the metabolic disturbance in pancreatic diabetes is due to an alteration in the balance between hepatic glycogenesis and glycogenolysis. The associated increase in protein metabolism is attributed to a compensatory acceleration of

oxidative deaminization in the liver in consequence of a decrease in the amount of carbohydrate available for utilization by the liver "per se".

We are indebted to Miss Dorothea Hamm for technical assistance and to the Eli Lilly Co. for generous supplies of sodium amytal.

REFERENCES

(1) Barker, S. B., W. H. Chambers and T. Dann. J. Biol. Chem. **118:** 177, 1937.
(2) Mirsky, I. A., J. D. Heiman and R. H. Broh-Kahn. This Journal **118:** 290, 1937.
(3) Mirsky, I. A. and R. H. Broh-Kahn. Ibid. **119:** 734, 1937.
(4) Mirsky, I. A. and R. H. Broh-Kahn. Ibid. **120:** 446, 1937.
(5) Wierzuchowski, M. J. Biol. Chem. **73:** 417, 1927.
(6) Deuel, H. J., Jr., H. E. C. Wilson and A. T. Milhorat. J. Biol. Chem. **74:** 265, 1927.
(7) Major, S. G. and F. C. Mann. This Journal **102:** 409, 1932.
(8) Bodo, R. C., F. C. CoTui and L. Farber. Ibid. **103:** 18, 1933.
(9) Soskin, S. J. Nutrition **3:** 99, 1930.
(10) Chaikoff, I. L. J. Biol. Chem. **74:** 203, 1927.
(11) Mann, F. C. and T. B. Magath. Arch. Int. Med. **31:** 797, 1923.
(12) Soskin, S., I. A. Mirsky, L. M. Zimmerman and N. Crohn. This Journal **114:** 110, 1935.
(13) Young, F. G. J. Physiol. **87:** P6, 1936.
(14) Edson, N. L. Biochem. J. **30:** 1862, 1936.

FACTORS WHICH INFLUENCE DARK ADAPTATION[1]

CHARLES A. ELSBERG AND H. SPOTNITZ

From the Clinical Research Laboratories of the New York Neurological Institute

Received for publication June 15, 1937

There have been many and careful studies of dark adaptation since that process was described by Aubert. Reliable measurements of foveal dark adaptation were made by Hecht who showed that at the fovea the process is extraordinarily rapid—being practically over in a few minutes.

During the course of our studies of the value of functional visual tests for the localization of tumors of the brain, a series of investigations was made of the time required for dark adaptation at the fovea. The studies were limited to one part of the dark adaptation curve,—the time which elapsed between the exposure of the dark adapted eyes to a bright light and the return of the ability to see a given test object.

The experiments were made in a dark room by means of the apparatus and procedure elsewhere described. Ten minutes were allowed for preliminary dark adaptation. The test objects were illuminated by red light and were viewed from a distance of 3.75 meters through a red Wratten filter (F 29) which excluded all spectral rays excepting those beyond 610$\mu\mu$.

The test objects that were used which have been described and illustrated in a paper already published, consisted of:

	TOTAL AREA	OUTER WHITE AREA	INNER BLACK AREA
	sq. mm.	*sq. mm.*	*sq. mm.*
1. A white square of 40 mm.	1600		
2. A 40 mm. square with internal contrast	1600	1200	400
3. A 40 mm. square with internal contrast	1600	800	800
4. A 40 mm. square with internal contrast	1600	400	1200
5. A 9 mm. white square	81		
6. A 9 mm. white square with internal contrast	81	32	49
7. A 6400 mm. square with internal contrast	6400	1600	4800

All of the test objects were on a black background and at the distance from which they were viewed were within foveal vision. The following series of binocular tests were made:

[1] Supported by a grant for brain tumor research from the John and Mary R. Markle Foundation.

I. After the minimum light intensity necessary for threshold vision of a test object had been established, the eyes were exposed from a distance of 40.5 cm. to a light intensity of 80 foot candles for 5 or 15 seconds (light adaptation). The time required for dark adaptation, i.e., until the observer could again see the test object *illuminated by threshold light* was ascertained.

II. The procedure was similar to I excepting that the intensity of illumination of the test object was 20 per cent, 50 per cent or 100 per cent *above the minimum required for threshold vision.*

TABLE 1

D.A. for two individuals after 5 seconds' exposure to bright light*

TEST OBJECT			C. A. E.			H. S.		
T.A.*	W.A.*	B.A.*	Threshold light	Bright light	Time required for dark adaptation	Threshold light	Bright light	Time required for dark adaptation
					seconds			*seconds*
1600	1600		4.0	80	15.1	3.5	80	11.9
81	81		12.9	80	15.1	7.2	80	14.2
81	32	49	17.5	80	15.3	11.4	80	13.3
6400	1600	4800	5.75	80	14.4	3.8	80	12.5

* D.A. = dark adaptation; T.A. = total area; W.A. = outer white area; B.A. = inner black area.

TABLE 2

D.A. after 15 seconds' exposure to bright light

TEST OBJECT			C. A. E.		
T. A.	W. A.	B. A.	Threshold light	Bright light	Time required for dark adaptation
					seconds
1600	1200	400	4.9	80	44.9
1600	800	800	5.75	80	46.6
1600	400	1200	9.05	80	46.3

III. Studies of the time required for dark adaptation with successively greater intensities of illumination of one test object.

IV. Studies of the time required for dark adaptation with progressive increase a, in the *intensity* of the bright light, and b, in the *time of exposure* of the eyes to the bright light.

V. Tests to determine the relative importance of the time and the intensity factors for dark adaptation.

In the tests described in I and II a series of test objects was used, and

in III, IV and V one test object was used. Each test was repeated at least three times and the results were averaged.

I. *The time required for dark adaptation at the threshold.* In these two series of tests the intensity of the bright light and the duration of exposure to it were constant. The illustrative protocols show that for any individual and a test object illuminated by threshold light, *the time required for dark adaptation was the same* (within the limits of experimental error)

TABLE 3

Time required for dark adaptation with the test object illuminated by light 20 per cent above the threshold

(5 seconds' exposure to the bright light)

TEST OBJECT			J. S.		
T. A.	W. A.	B. A.	Illumination of test object	Bright light	Time required for dark adaptation
					seconds
1600	1600		5.55	80	10.8
1600	1200	400	6.6	80	10.8
1600	800	800	6.8	80	11.4
1600	400	1200	9.7	80	10.4

TABLE 4

Time required for dark adaptation with the test object illuminated by light 50 per cent above the threshold

(Eyes exposed to bright light for 5 seconds)

TEST OBJECT			ILLUMINATION OF TEST OBJECT	BRIGHT LIGHT	TIME REQUIRED FOR DARK ADAPTATION
T. A.	W. A.	B. A.			
					seconds
1600	1600		7.0	80	6.0
1600	1200	400	8.8	80	5.6
900	500	400	13.9	80	6.2
6400	1600	4800	6.6	80	5.7

no matter what the area of the test object or whether the object was a white square or a square with internal contrast. (Tables 1 and 2.)

II. *The time required for dark adaptation when the test objects were illuminated by light intensity above the threshold.* In one series, each test object was illuminated by a light which was 20 per cent above the minimum intensity required for threshold vision; in another series of tests the light was 50 per cent, and in a third series, 100 per cent above the threshold. A few illustrative records of the results of the tests follow: (Tables 3, 4, 5).

These tests showed that with a constant intensity of the bright light

and equal duration of the exposure to it, *the time required for dark adaptation was the same for all of the test objects that were used in any one series in which the test object was illuminated by threshold light or by light which was 20 per cent, 50 per cent or 100 per cent above the threshold.*

III. *The time required for dark adaptation with successive increases in the intensity of illumination of the test object.* The results of the tests showed that the refractory period became shorter as the intensity of illumination of the test object was increased. Similar results have been published by a number of investigators (Graham, Geldard, Lythgoe and Tansley, Karn, Dorothy Adams, etc.).

IV. *The time required for dark adaptation with successive increases in the time of exposure to the bright light or in the intensity of the bright light.* The tests were made with a white square of 9 mm. as the test object. Either

TABLE 5

Time required for dark adaptation with the test object illuminated by light 100 per cent above the threshold

(Eyes exposed to bright light for 5 seconds)

TEST OBJECT			ILLUMINATION OF TEST OBJECT	BRIGHT LIGHT	TIME REQUIRED FOR DARK ADAPTATION
T. A.	W. A.	B. A.			
					seconds
1600	1600		10.4	80	4.5
1600	1200	400	10.8	80	5.4
900	500	400	13.6	80	5.1
6400	1600	4800	10.8	80	5.1

the duration of the exposure to the bright light was progressively lengthened or the intensity of the bright light was increased.

Three series of tests on two individuals are shown in figure 1. In these tests the length of exposure to the bright light was progressively increased. In one series (A, B) the test object was illuminated by a light of 12 units; in the second (A′, B′) by a light of 22 units, and in the third, by a light of 25 units (foot candles). The graph shows that the time required for dark adaptation became longer as the duration of exposure to the bright light was increased. Furthermore, the refractory period was shorter when the illumination of the test object was greater. When the test object was illuminated by 25 units of light (about 200 per cent above the threshold) the refractory period was shorter for any length of exposure to the bright light than when the illumination of the test object was 22 or 12 units.

Tests in which the duration of exposure to the bright light was kept the same but the intensity of the light was progressively increased, showed a similar lengthening of the time required for dark adaptation. The results of the tests were in agreement with the conclusions arrived at by

other investigators that an increase in the intensity of the bright light or a longer exposure of the eyes to it, causes a prolongation of the time required for dark adaptation.

The conclusions from the tests described in III and IV may be stated thus: *The time required for dark adaptation becomes less as the difference between the intensity of the light by which the test object is illuminated and the intensity of the bright light to which the eyes are exposed is diminished.* Fur-

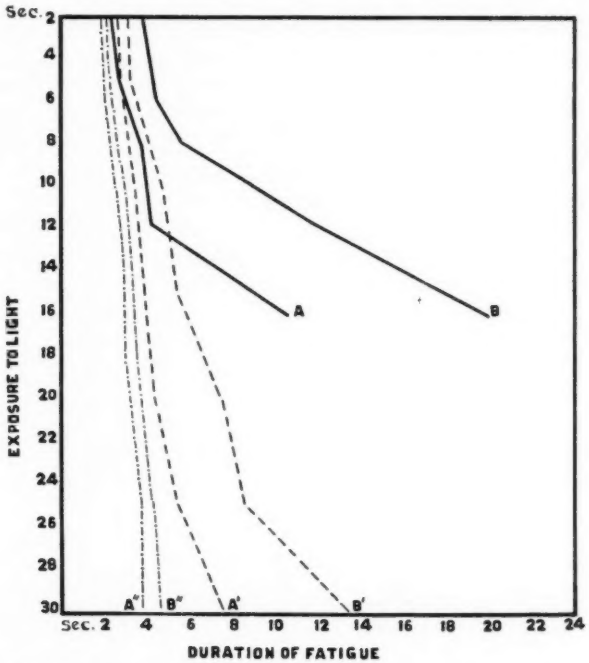


Fig. 1. The time required for dark adaptation for two individuals (A, B; A′B′; A″B″) with progressive increase in the exposure of the two eyes to a bright light. The continuous lines indicate the duration of the refractory period for the two individuals when the test object was illuminated by light of 12 foot candles; the broken lines when the illumination was 22 foot candles; and the broken and dotted lines when the illumination was 25 foot candles.

thermore the time required for dark adaptation becomes less as the duration of the exposure to the brighter light is diminished.

V. *The relative importance of the time and intensity factors in dark adaptation.* The experiments of Johannsen showed that the two factors of time and intensity are not interchangeable in producing the characteristic of the eye which determines the subsequent dark adaptation.

In order to learn which factor was more important, a series of tests were

made which were based upon the following considerations. According to the Bunsen-Roscoe law IT = C, i.e., the total quantity of light which reaches the eyes is constant with a constant variation of either intensity or time. For example, the total quantity of light is the same whether a light of 80 units of intensity acts for 15 seconds (80 × 15 = 1200); one of 60 units acts for 20 seconds (60 × 20 = 1200); one of 40 units for 30 seconds (40 × 30 = 1200); and so on. In the same way, the number of light intensity units is 600, when I = 80 and T = 7.5″; I = 60 and T = 10″; I = 40 and T = 15″; or I = 20 and T = 30″.

The test object was a black and white square whose total area was 6400 sq. mm. in which the outer white area was 1600 sq. mm. and the inner black area 4800 sq. mm. The test object was illuminated by light whose intensity was 20 per cent above that necessary for threshold vision. The eyes were exposed to light of varying intensity for varying periods of time according to the law that I × T = C, and the time required for dark adaptation was ascertained for three individuals.

	I	T	I × T	TIME REQUIRED FOR DARK ADAPTATION (SECONDS)	
				C. A. E.	*H. S.*
1. I.T. = 50	1.25	40	50	7.3	12.1
	1.60	30	50	4.6	8.7
	2.50	20	50	3.2	6.7
	5.0	10	50	2.5	4.0
				C. A. E.	*J. S.*
2. I.T. = 200	3.3	60	200	11.4	10.9
	6.6	30	200	9.5	7.4
	10.0	20	200	6.9	7.2
	20.0	10	200	4.9	5.0
				C. A. E.	*H. S.*
3. I.T. = 400	6.6	60	400	23.5	28.6
	13.3	30	400	14.1	15.4
	20.0	20	400	11.1	12.6
	40.0	10	400	6.7	8.9
4. I.T. = 600	10	60	600	25.3	29.7
	20	30	600	16.8	23.4
	40	15	600	13.9	17.8
	60	10	600	10.5	14.3
				C. A. E.	*J. S.*
5. I.T. = 1200	20	60	1200	31.5	24.2
	40	30	1200	23.3	17.5
	60	20	1200	16.6	12.9
	80	15	1200	15.6	10.4
				C. A. E.	*H. S.*
6. I.T. = 1600	20	80	1600	33.4	34.4
	40	40	1600	23.3	29.4
	80	20	1600	19.0	21.2
	260	6.2	1600	7.3	12.0

However, the effect upon nerve cells and the resulting *sensations* are not the same when the eyes are exposed to a light stimulus of high intensity for a short time as when they are exposed to a light stimulus of lower intensity for a longer period. This was demonstrated by the results of a series of tests which allowed one to arrive at a conclusion regarding the relative importance of time and intensity in dark adaptation.

In this series of tests, the time required for dark adaptation was studied when $I \times T = 50, 200, 400, 600, 1200$ or 1600 light intensity units.

These tests (see page 694) demonstrated the interesting fact that the duration of the refractory period, i.e., the time required for dark adaptation—was always longest when the intensity of the bright light was least and the duration of exposure to it the longest.

The results of each series of tests in which $IT = C$ showed that the *exposure of the eyes to a light of relatively low intensity for a relatively long period of time caused a change which was recovered from more slowly than when the eyes were exposed to a light of relatively high intensity for a relatively short period of time.* Therefore, the conclusion must be that, for the effect of the bright light on the time of dark adaptation, time is of more importance than intensity.

It would be of no little interest if the tests described in this paper concerning the factors which influence dark adaptation should be used for the study of action currents in the retino-cerebral structures. By this means it might be possible to gain a deeper insight into the functional activity of the retina and of the visual pathways and centers and their responses to the stimulus of light.

SUMMARY

1. The time required for dark adaptation was the same for each individual, for the various test objects each illuminated by threshold light, as long as the intensity of the bright light and the duration of exposure to it were constant.

2. The same was the case when each test object was illuminated by light 20 per cent, 50 per cent or 100 per cent above the threshold.

3. The time required for dark adaptation became shortened as the difference between the intensity of the light by which the test object was illuminated and the intensity of the bright light to which the eyes were exposed was diminished.

4. For the time required for dark adaptation, the time during which the eyes are exposed to the bright light (light adaptation) is of more importance than the intensity of the bright light.

REFERENCES

ELSBERG, C. A. AND H. SPOTNITZ. Bull. Neurol. Inst. N. Y. **6**: no. 2, 1937.
JOHANNSEN, D. E. Gen. Physiol. **10**: 1, 1934.

SERUM AND URINE PHOSPHATASE ACTIVITY IN THE CAT AFTER LIGATION OF THE COMMON BILE DUCT

CHARLES A. FLOOD, ETHEL BENEDICT GUTMAN AND ALEXANDER B. GUTMAN

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City

Received for publication June 16, 1937

The evidence now available suggests that the phosphatase present in serum arises in large part in the bones, whence it passes into the blood, traverses the liver and is excreted with the bile into the intestinal tract. This view is based chiefly upon the presence of large concentrations of a phosphatase in bones and bile which appears to be identical with the phosphatase in serum (1, 2, 3). In accord with this concept, it has been shown that ligation of the common bile duct in the dog results in a marked rise in phosphatase activity of the serum (2, 4). In man, obstruction of the common bile duct is likewise associated with increased phosphatase activity of the serum, a finding which has been utilized in the differential diagnosis of jaundice (5).

Ligation of the common bile duct in the *cat*, however, as reported recently by Cantarow, Stewart and McCool (6, 7) does *not* cause a significant rise in the phosphatase activity of the serum; a result which appears to be inconsistent with prevailing views of phosphatase metabolism in man.

In confirmation of Cantarow, Stewart and McCool, our own results also show no striking rise in serum phosphatase activity after ligation of the common bile duct in the cat. We find, however, that the urine of the cat normally contains considerable amounts of a phosphatase which, like serum phosphatase, is active at pH 9.0. Moreover, the amount of this enzyme excreted in the urine tends to increase—at least transiently—after ligation of the common bile duct. In the dog and in man, the urine does not contain significant amounts of phosphatase active at pH 9.0 either under normal conditions or following obstruction of the common bile duct (2, 8).

We have not been able to establish that the phosphatase found in the urine of the cat came from the blood, since kidney tissue, which contains appreciable amounts of "alkaline" phosphatase, could not be excluded as a source. It seems likely, however, that the cat normally excretes serum phosphatase through the kidney as well as through the biliary tract. Urinary excretion of serum phosphatase in the cat would explain the

absence of increased serum phosphatase activity after common bile duct ligation—as contrasted with the rise regularly observed in man and the dog, whose kidneys appear to be impermeable to phosphatase.

METHODS. Twenty-one cats weighing from 2.9 to 5.2 kgm. were used in this study. The animals were maintained on a routine diet consisting chiefly of meat, milk, bread and vegetables. Ligation and severance of the common bile duct was performed on twelve animals, the duct being divided about 0.5 cm. above its entrance into the duodenum. Preoperative and postoperative studies of blood and urine were carried out, about 5 cc. of blood being obtained by cardiac puncture and the urine being secured by catheterization. The animals were sacrificed and autopsied at intervals ranging from 5 to 52 days after operation. Nine additional animals were used to obtain control values of serum, urine and bile phosphatase.

Serum phosphatase activity was determined on 0.5 cc. samples by Bodansky's method (9), the results being expressed in Bodansky units.[1] Inorganic phosphorus was determined in serum by the method of Kuttner and Lichtenstein, as modified by Bodansky (11), non-protein nitrogen by the micro-Kjeldahl technique. Urine phosphatase activity was determined by the King and Armstrong method (12) at 37° using disodium-monophenylphosphate as substrate. Sodium veronal was employed as a buffer for determinations at pH 9.0 and Soerensen's citrate buffer for determinations at pH 5.0. Urine was diluted 5 to 10 times and hydrolysis was carried out for ½ to 5 hours, depending upon the amount of phosphatase found in preliminary estimations. The results are recorded in King and Armstrong units, as calculated for ½ hour. Determinations of the phosphatase activity of bile, obtained at autopsy, were made by the same method. Serum bilirubin was determined by the method of Thanhauser and Andersen.

Ultrafiltration of urine samples was performed in a Simms surge ultrafiltration apparatus (13), using collodion membranes, at positive pressures of 40 to 50 mm. Hg.

RESULTS. *Serum.* (Table 1.) The serum phosphatase activity in 19 control observations on 18 cats ranged from 0.6 to 5.0 Bodansky units per 100 cc. serum. After ligation of the common bile duct, the serum phos-

[1] No correction was made in jaundiced animals for the inhibitory effect of bile acids upon the activity of serum phosphatase (10, 3) because no definite inhibition could be demonstrated. It was found that the phosphatase activity of equal parts of jaundiced cat serum and normal cat serum or of jaundiced cat serum and serum of a patient with Paget's disease closely approximated the mean of the respective phosphatase activities. Apparently, the concentration of bile acids in the serum of these animals was insufficient to be effective. The bile acid content of serum is known to fall relatively early after an initial rise following ligation of the common bile duct.

TABLE 1

Serum, urine and bile phosphatase activity of the normal cat and after ligation of the common bile duct

NUMBER	DATE	SERUM				URINE PHOSPHATASE pH 9.0 (KING AND ARMSTRONG UNITS PER 100 CC.)
		Inorganic P	Phosphatase (Bodansky units 100 cc.)	NPN	Bilirubin	
1. Control animals						
		mgm. per cent		*mgm. per cent*	*mgm. per cent*	
1–9		3.9–5.5	1.1–4.0	36–43		0.5–19.0
2. Operated animals						
10	12- 9-36					5.8
	12-15-36	5.5	5.0			6.3
	12-17-36	Operation: Ligation common bile duct				
	12-19-36					6.6
	12-24-36	7.0	9.0		4.5	14.2
	1- 5-37	8.0	2.9		4.3	2.0
	1-13-37	Operation: Ligation both ureters				
	1-15-37	11.1	7.5	125	5.0	
11	1- 7-37					22.0
	1- 8-37	5.5	1.5	41		17.0
	1-12-37	Operation: Ligation common bile duct				
	1-19-37	5.5	4.0	47	5.8	60.0
	1-27-37	8.6	1.6	50	3.2	
12	1- 7-37					2.5
	1- 8-37	5.0	0.6	33		8.7
	1-12-37	Operation: Ligation common bile duct				
	1-19-37	7.5	0.8	58	3.2	1.5
	1-27-37	6.1	3.6	53	8.0	34.2
13	2- 3-37					3.8
	2- 8-37	5.0	0.8	40		1.4
	2- 9-37	Operation: Ligation common bile duct				
	2-23-37	6.7	5.7	47	3.8	−1.2*
14	2-15-37	5.3	1.0			−0.9*
		Operation: Ligation common bile duct				
	2-19-37	4.9	4.7	38	3.2	12.4
	3- 5-37	7.7	15.7	69	16.6	71.0
15	2-17-37	9.6(?)	4.1	48		0
	2-17-37	Operation: Ligation common bile duct				
	2-19-37	7.8	2.8	43	0	4.6
	3-12-37	7.6	3.9	59	9.9	52.0
	3-24-37	6.9	3.2	45	14.4	20.4

* In the presence of large excesses of phosphate, the reaction goes in the direction of phosphate formation.

TABLE 1—*Concluded*

NUMBER	DATE	SERUM				URINE PHOSPHATASE pH 9.0 (KING AND ARMSTRONG UNITS PER 100 CC.)
		Inorganic P	Phosphatase (Bodansky units 100 cc.)	NPN	Bilirubin	
2. Operated animals—*Concluded*						
		mgm. per cent		*mgm. per cent*	*mgm. per cent*	
16	2-24-37	3.0	4.9			15.2
	2-27-37	4.8	1.7	42		
	3- 3-37	Operation: Ligation common bile duct				
	3- 5-37	4.8	2.9	48	3.2	73.5
	3-23-37					57.3
	3-29-37	6.3	1.8	57	5.8	6.4
17	3- 3-37	5.4	3.2	42		13.3
	3-10-37	Operation: Ligation common bile duct				
	3-12-37	5.2	1.6	43	0	
	3-24-37	6.8	3.1	59	2.1	−0.6*
18	3-10-37	6.2	1.5	33		0.9
	3-12-37	Operation: Ligation common bile duct				
	3-23-37					−1.5*
	3-31-37					19.6
19	3-10-37	4.5	2.0	30		23.3
	3-24-37	Operation: Ligation common bile duct				
	3-31-37	6.2	2.7	56	5.8	8.0
	4-13-37	4.6	2.8	37	12.1	18.5
	5-10-37	3.8	5.6	32	8.7	15.5
	5-13-37	Operation: Ligation both ureters				
	5-14-37	10.6	4.3	110	10.0	
	5-15-37	17.1	3.4	168	8.5	11.5†
20	3-15-37	5.7	2.2	34		20.7
	3-16-37	Operation: Ligation common bile duct				
	4- 6-37	4.6	7.3	59	13.2	53.0
	4-20-37	3.6	5.6	41	7.5	96.5
	5- 4-37	4.3	12.9	31	6.6	10.8
21	3-16-37	4.5	1.9	28		30.5
	4- 2-37	Operation: Ligation common bile duct				
	4-13-37	4.3	11.2	50	3.4	5.7

† Urine from kidney pelvis.

phatase activity rose above the level found preoperatively in 8 of 11 animals studied. This rise was never striking, the highest value recorded in our series being 15.7 Bodansky units; and in only 7 of 23 post-operative

determinations of serum phosphatase activity was our normal maximum (5.0 Bodansky units) exceeded. Such post-operative elevations in serum phosphatase activity as occurred were usually transient and appeared to be unrelated to the post-operative time interval or to the severity of jaundice.

The non-protein nitrogen rose postoperatively in all of the 9 animals studied. This rise was not marked, however, the highest value encountered being 69 mgm. per cent. In two animals, subsequent ligation of both ureters (for reasons to be considered presently) resulted in non-protein nitrogen figures as high as 168 mgm. per cent. Associated presumably with the nitrogen retention, hyperphosphatemia was found in most instances.

Urine. (Table 1.) A phosphatase which is active at pH 9.0 was found in the urine in 17 of 19 normal cats studied. The urine phosphatase activity varied widely under normal conditions not only in different animals but also in the same cat at different times. After ligation of the common bile duct, the "alkaline" phosphatase activity of the urine rose above the pre-operative level on at least one examination in eight of twelve cats studied. In five of these animals, the post-operative level exceeded our maximum value (30.5 King and Armstrong units) for normal cat urine. The highest post-operative value observed was 96.5 King and Armstrong units per 100 cc. urine.

The urine of the normal cat also contains small amounts (up to 3.5 King and Armstrong units) of a phosphatase which is active at pH 5.0. This enzyme was found in female as well as male animals. Ligation of the common bile duct did not affect the "acid" phosphatase activity of the urine significantly in three cats investigated.

Bile. The bile of two normal animals was found to contain 49 and 134 King and Armstrong units of phosphatase active at pH 9.0. After ligation of the common bile duct, the phosphatase activity of bile appears to decrease. In two cats examined five and seven weeks respectively after common bile duct ligation, bile obtained from the gall bladder exhibited no phosphatase activity whatever. In one instance (cat 19), the bile was also devoid of pigment ("white bile") and contained no cholesterol.

Discussion. In view of the presence of "alkaline" phosphatase in normal cat urine, escape of phosphatase in the urine offers a plausible explanation for the absence of appreciable rises in serum phosphatase activity after ligation of the common bile duct. In support of this view, the phosphatase activity of the urine of most of our animals was higher after operation than pre-operatively. This post-operative rise in urine phosphatase activity however was usually transient and inconstant resembling in this respect the behavior of other bile constituents appearing in the urine after common duct obstruction.

We were unable to demonstrate increased serum phosphatase activity

after ligation both of the common bile duct and of the ureters (cats 10 and 19, table 1). Other factors such as decreased production or increased deposition of phosphatase in the tissues may, however, play a rôle in lowering the level of serum phosphatase activity under such conditions.

The failure of serum phosphatase activity to increase after common bile duct ligation in the cat might be attributed to the accumulation of inhibitory substances such as bile acids in the serum. This alternative explanation seems, however, unlikely since there is no evidence that such substances are present in cat serum in greater concentration than in human or dog serum. The further possibility that the liver contributes to serum phosphatase formation (14) and that production is inhibited by common bile duct ligation deserves mention. No evidence on this point is available.

Since serum phosphatase is either a protein or a substance bound to protein (1), it was important to ascertain whether or not the "alkaline" phosphatase found in cat urine would pass through a semi-permeable membrane. It was found that ultra-filtrates of cat urine obtained both before and after ligation of the common bile duct were consistently devoid of phosphatase activity.

SUMMARY

1. The phosphatase activity of serum, urine and bile was studied before and after ligation of the common bile duct in the cat. In eight of eleven animals, a slight rise in serum phosphatase activity occurred after common bile duct ligation. This rise is negligible in comparison with the marked elevations regularly observed following obstruction of the common bile duct in the dog and in man.

2. It was found that the urine of the cat, unlike that of man and the dog, normally contains considerable amounts of a phosphatase which is active at pH 9.0. The excretion of this enzyme in the cat tends to increase transiently after ligation of the common bile duct.

3. It seems likely that the cat, unlike man and the dog, normally excretes serum phosphatase through the kidney as well as through the biliary tract. Urinary excretion of serum phosphatase in the cat may explain the absence of increased serum phosphatase activity after ligation of the common bile duct.

REFERENCES

(1) Subject reviewed recently by S. J. Folley and H. D. Key. Erg. Enzymforschung **5**: 159, 1936.

(2) Armstrong, A. R., E. J. King and R. I. Harris. Canadian M. A. J. **31**: 14, 1934.

(3) Bodansky, O. J. Biol. Chem. **118**: 341, 1937.

(4) Bodansky, A. and H. L. Jaffe. Proc. Soc. Exper. Biol. and Med. **31**: 1179, 1934.

(5) Subject reviewed recently by C. A. Flood, E. B. Gutman and A. B. Gutman. Arch. Int. Med. **59:** 981, 1937.
(6) Cantarow, A., H. L. Stewart and S. G. McCool. This Journal (Proc.) **113:** 25, 1935.
(7) Cantarow, A., H. L. Stewart and S. G. McCool. Proc. Soc. Exper. Biol. and Med. **35:** 87, 1936.
(8) Flood, C. A., E. B. Gutman and A. B. Gutman. Unpublished data.
(9) Bodansky, A. J. Biol. Chem. **101:** 93, 1933.
(10) Takata, H. J. Biochem. Japan **16:** 83, 1932.
(11) Bodansky, A. J. Biol. Chem. **99:** 197, 1932.
(12) King, E. J. and A. R. Armstrong. Canadian M. A. J. **31:** 376, 1934.
(13) Simms, H. S. and N. P. Stillman. J. Gen. Physiol. (in press).
(14) Bodansky, A. and H. L. Jaffe. Proc. Soc. Exper. Biol. and Med. **31:** 107, 1933.

THE GALVANIC NYSTAGMUS REACTION IN THE MONKEY

PAGE NORTHINGTON AND S. E. BARRERA

From the Department of Neuropathology, New York State Psychiatric Institute and Hospital, New York, N. Y.

Received for publication June 19, 1937

Numerous observations have been made clinically and in the experimental laboratory on nystagmus induced by the galvanic current. These phenomena were studied in monkeys by Uffenorde (1) and Blau (2). Despite these many observations, no conclusive results as to the participation of the eighth nerve in the response in monkeys has been presented.

Macacus rhesus monkeys, averaging 10 pounds in weight, in all twenty-four animals, were used for the experiments. The current employed was obtained from an ordinary clinical galvanic testing battery equipped with variable resistance, milliameter and polarity-changing switch. Leads from the battery were used in two ways in order to elicit the response.

In ear to ear examinations the tips of the leads, wrapped in cotton and immersed in normal saline solution, were wedged into the external auditory canals. The polarity of the individual leads could be changed at will by the polarity-changing switch. With this technique both ears were tested essentially at the same time. To test the individual ears predominantly, the ear to back and back to ear records were made. One electrode, instead of being placed in an ear, was attached to a flat electrode about 7 cm. in diameter, which was immersed in saline solution and held firmly on the shaved back of the monkey between the scapulae. Using these two methods it was found that in the normal monkey a current of 1 to 3 m.a. produced a normal type, horizontal nystagmus with the electrodes in both ears. With the ear to back technique it was found that in the animal a current of 2 to 4 m.a. produced a normal type nystagmus with the cathode in the ear and the anode on the back. With polarities reversed, 4 to 5 m.a. were required. The direction of the nystagmus elicited (quick component) was always toward the cathode with the electrodes in the two ears and away from the anode with the anode in the ear and cathode on the back.

Observations were made on two types of experimental animals. One type consisted of monkeys in which unilateral labyrinthectomy was performed (Hinsberg technique). Some cases resulted in degeneration of the trunk of the eighth nerve, others did not. In the cases in which the nerve

remained undegenerated, despite a clinically non-functioning labyrinth which gave no response to caloric testing, the galvanic response could be obtained from ear to ear, ear to back and back to ear with essentially normal values in current. On the other hand, in those cases in which, as a result of labyrinthectomy degeneration of the eighth nerve occurred, the galvanic response with the cathode in the labyrinthectomized ear occurred only with a somewhat increased current intensity (5 to 7 m.a.) and the ear to back and back to ear tests failed to produce a nystagmus response on the labyrinthectomized side, even with current intensities up to 20 m.a. The operative lesions and the resulting nerve degenerations were controlled by serial sections of the temporal bones stained by the H. and E. and the P.T.A.H. methods and serial sections of the brain stem prepared by the Weigert myelin sheath method.

To control this observation a second group of monkeys were studied in which the eighth nerve was sectioned intracranially. In these cases the galvanic response always failed to appear on the side with the nerve sectioned as tested by the ear to back and back to ear techniques even with current intensities up to 20 m.a. In the ear to ear tests such animals required again a moderate increase in the current intensity (5 to 7 m.a.) when the cathode was placed in the ear on the side of the sectioned nerve.

The results indicate that an anatomically or functionally intact labyrinth is not necessary in the production of nystagmus by the galvanic current since a normal type nystagmus occurs even with a destroyed labyrinth. On the other hand, an intact eighth nerve would appear to be necessary for eliciting the response because the test is negative when the nerve trunk is severed or when its degeneration results from certain labyrinthectomies. Clinically, the test would seem to be of value in distinguishing nerve degeneration in cases where the galvanic or rotation tests indicate peripheral vestibular dysfunction. Indeed, we feel that a positive galvanic response in the presence of a negative caloric response on the side of vestibular dysfunction indicates that the eighth nerve is not degenerated on that side.

REFERENCES

(1) Uffenorde, W. Münch. Med. Wchnschr. **59**: 1213, 1912.
(2) Blau, A. Verhandl. der d. otologisch. Gesell. **22**: 208, 1913.

EFFECTS OF MECHOLYL ON GASTRIC SECRETION

JOHN S. GRAY AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois

Received for publication June 19, 1937

Dale and Feldberg (1) have shown that acetylcholine may be demonstrated in blood flowing from the stomach in the period during which the vagus nerves to that organ are being stimulated electrically. Vineberg (2) is among the most recent investigators to report that stimulation of the vagus nerves elicits a flow of acid gastric juice. One would anticipate on the basis of these observations that the injection of acetylcholine or some related compound would be effective in stimulating a similar flow of gastric juice. In fact, Suda (3) reported acetylcholine and Nöll (4) carbaminoylcholine to be effective in stimulating acid secretion in dogs. Masek (5) and Gavazzani (6) have made similar observations in human subjects. However, several authors (7, 8, 9, 10) have reported that choline compounds inhibit as frequently as they stimulate acid secretion. Da Costa (11) observed no stimulation of any kind, and Ferguson and Smith (12) and Myerson, Rinkel and Dameshek (13) obtained a flow of alkaline gastric fluid. In view of these contradictory reports we have reinvestigated the problem. We also desired to ascertain whether histamine and choline esters stimulated the parietal cell by exciting the same mechanism.

METHODS. Mecholyl (acetyl-β-methylcholine chloride) was selected for this study because it is a comparatively potent and stable choline ester which lacks ganglionic action (14) (15). In order to be sure that the results obtained were not peculiar to this drug, certain of the more significant experiments were repeated, substituting acetylcholine for mecholyl.

Four dogs having pouches of the entire stomach were used throughout this work. The pouches had been prepared from three months to two years before these experiments were undertaken. Since this type of gastric pouch lacks vagus innervation, certain of the experiments were repeated on Pavlov pouch dogs in order to be certain that the absence of the vagus nerves was in no way influencing the results. The Pavlov pouch dogs were typical in that to a meal of meat the maximum volume of secretion was obtained during the first hour.

Because of previous rather extensive experience (16) in obtaining a continuous flow of gastric juice in these animals by regularly repeated injections of histamine, this same type of procedure was applied to the study of mecholyl. This drug was injected subcutaneously in small quantities every ten minutes. The gastric juice was titrated *in toto* with N/10 NaOH using Topfer's and phenolphthalein as indicators.

RESULTS. 1. *Continuous mecholyl secretion.* Figure 1 shows the curves of secretion obtained by injecting mecholyl subcutaneously every ten minutes in total pouch dogs. Each curve throughout this paper represents the average of four experiments, one on each of the four dogs. It will be noted in the curves of figure 1 that all reach a maximum, which is followed by a decline, even though the mecholyl injections were continued

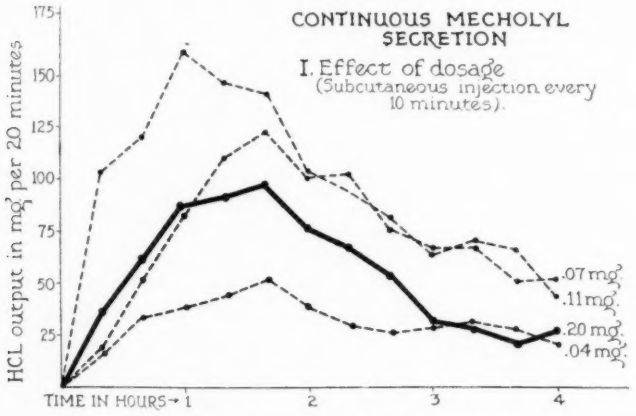


Fig. 1. Gastric secretion stimulated by various doses of mecholyl injected subcutaneously every ten minutes. Note that the largest dose (heavy line) does not give the greatest stimulation.

throughout the experiment. This is in contrast to the results obtained with histamine which produces a plateau of secretion which persists practically indefinitely. The acidity of the mecholyl secretion is as high as that of histamine secretion. The second point of interest in figure 1 is that as the dosage of mecholyl increases from 0.04 mgm. to 0.11 mgm. every ten minutes, the peak of the curve becomes higher, occurs earlier, and is followed by a greater decline. When the dosage reached 0.20 mgm., however, the peak of the curve is definitely lower than in the other curves. This shows that there is an optimum dosage and suggests that a dosage reversal effect exists.

It became of interest to know whether the decline of mecholyl secretion

was accompanied by a state of insensitivity to other gastric excitants. In figure 2 it is shown that 0.5 mgm. of histamine injected subcutaneously in a single dose at the height of mecholyl secretion greatly augmented the rate of secretion. The same dose, however, injected after the decline of secretion had become marked was practically without effect. The same results were obtained when acetylcholine was substituted for the mecholyl, indicating that it is probably characteristic of choline esters in general to render the gastric glands refractory, even to histamine.

Figure 2 also shows that 1 mgm. of atropine sulphate given subcutaneously can completely abolish mecholyl secretion.

2. *Effects of mecholyl on continuous histamine secretion.* The results so far presented suggest that mecholyl exhibits a dosage reversal effect. This could not be shown clearly by the above methods because larger doses

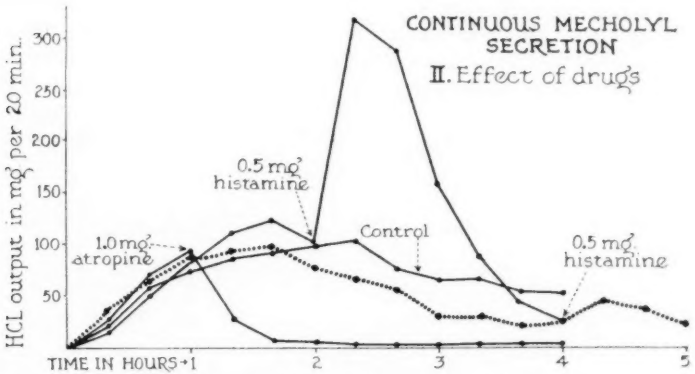


Fig. 2. The effect of repeated mecholyl injection on the gastric response to 0.5 mgm. histamine, and the inhibitory effect of 1.0 mgm. atropine on mecholyl secretion.

could not be injected continuously with safety. Contractions of the pylorus and pyloric antrum, which compose the fistula in these animals, became so vigorous under the influence of large doses of mecholyl that the mucosa was injured where it touched the collecting device. This resulted in bleeding sufficient to obscure changes in acidity of the gastric juice. Accordingly, the problem was attacked from another angle. Histamine was injected subcutaneously every ten minutes until a constant rate of acid production had been reached. The control curve in figure 3 shows that such a level can be maintained for hours without significant change. In one series of experiments 0.1 mgm. of mecholyl was administered in a single subcutaneous dose after histamine secretion had been established, and in other series 1.0 mgm. of mecholyl was similarly injected. Figure 3 shows that the small dose produced a marked increase in the rate of secre-

tion, whereas the large dose produced a marked inhibition of the histamine secretion. This is definite proof of the existence of a dosage reversal effect with mecholyl.

It became of interest to know whether both the excitatory and inhibitory actions of mecholyl could be abolished by atropine. The effect of atropine

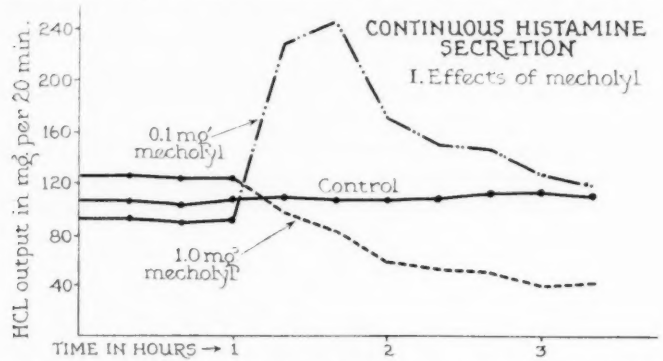


Fig. 3. Stimulation and inhibition of continuous histamine secretion with 0.1 mgm. and 1.0 mgm. mecholyl respectively.

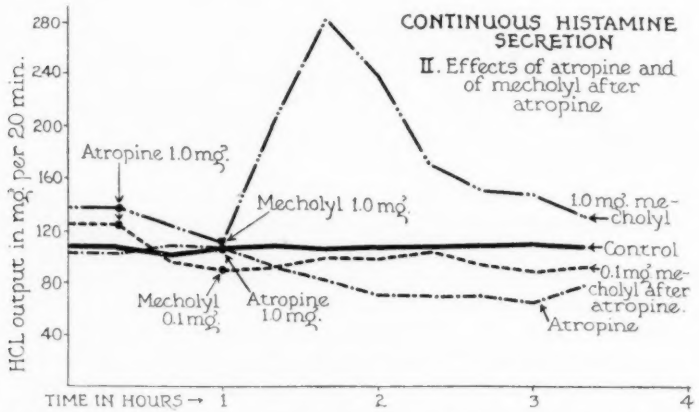


Fig. 4. The action of atropine against continuous histamine secretion, and against excitatory and inhibitory doses of mecholyl.

alone on continuous histamine secretion had previously been determined in these dogs and formed the substance of an earlier publication (20). Figure 4 shows that 1.0 mgm. of atropine exerts a definite but limited inhibitory action against histamine. In one series of experiments a high rate of continuous histamine secretion was established and 1.0 mgm. of atropine was

then injected. Forty minutes later, when the atropine had manifested its effect, 0.1 mgm. of mecholyl was administered. In another series the same procedure was repeated except that 1.0 mgm. of mecholyl was injected. As will be noted in figure 4, the excitatory effect of the 0.1 mgm. dose was abolished by the atropine and the inhibitory effect of the 1.0 mgm. dose was converted to an excitatory effect quantitatively similar to that previously obtained with 0.1 mgm. without atropine. This suggests that nine-tenths of the large dose of mecholyl was counteracted by the atropine, leaving only one-tenth to manifest its typical stimulation. In one trial, 2.0 mgm. of atropine were found to prevent any effect from 1.0 mgm. of mecholyl. One can conclude, therefore, that both the excitatory and inhibitory actions of mecholyl can be completely abolished by atropine.

The data presented show the effects of the above procedures on the production of HCl only. The volume changes throughout parallel the changes in total acid, with the exception that as inhibition became marked the volume fell somewhat less than the total acid so that the acidity was slightly decreased. However, only rarely did the acidity fall below N/10 HCl. These facts prove conclusively that the inhibition of acid was not due to neutralization by non-acid secretions, since this would entail an *increase* in volume of juice secreted in order to include the neutralizing and diluting fluid.

It should also be mentioned that the results indicate that mecholyl chloride is more potent than histamine acid phosphate on the basis of weight. However, molecule for molecule the two drugs are of approximately equal potency. It was found that mecholyl chloride is from 100 to 200 times more potent in stimulating gastric secretion than is acetylcholine when the two are given subcutaneously. However, if the acetylcholine is made up in a solution containing eserine, it becomes as potent as mecholyl. The difference in potencies is therefore due to the greater ease with which acetylcholine is hydrolized in vivo by choline esterase.

Discussion. It is important for the chemical theory of neuromuscular and neuro-glandular transmission, that the effects of nerve stimulation be reproducible by suitable administration of the chemical substance believed to be liberated by activity of the nerve. This study shows that the action of the vagus nerve on the parietal cell can be duplicated by the injection of acetylcholine and related compounds.

The phenomenon of dosage reversal noted in this study is not new in the field of chemical mediation by acetylcholine. Feldberg and Vartiainen (17) while investigating chemical mediation across sympathetic ganglia found that large doses of acetylcholine or eserine paralyzed synaptic transmission, whereas small doses had the reverse effect. In this they have been recently confirmed by Cannon and Rosenblueth (18). The dosage reversal effect will probably explain the previous contradictory reports

concerning the effect of choline compounds on gastric secretion. An examination of the literature (3–12) will show that those investigators who used large doses of acetylcholine or mecholyl failed to obtain a stimulation of acid secretion, whereas those who used small doses obtained this effect. Myerson, Rinkel and Dameshek (13) and Ferguson and Smith (12) gave extremely large doses of mecholyl, which produced marked side reactions such as salivation, sweating and circulatory disturbances. On several occasions large doses of mecholyl (0.5 to 1.0 mgm. every ten minutes) were given to one of the total pouch dogs. Although the results were obscured by the presence of blood in the gastric juice, no acid was apparently secreted. The juice was of small volume, very thick and viscous, consisting principally of mucus. Such doses, and in fact all supra-optimal doses, produced salivation in the animals whereas optimal and sub-optimal doses were without such action. This suggests that in dogs, as in humans and monkeys, doses of mecholyl which are so large as to produce marked side actions not only inhibit acid production but stimulate a flow of alkaline mucus.

This study leads to certain conclusions in regard to the site of action of the drugs involved. At first glance it would appear that mecholyl, in producing both its excitatory and inhibitory effects, acts on the same site within the parietal cell, namely, that mechanism concerned with receiving parasympathetic stimuli, because both of the effects are abolished by atropine. However, if this reasoning be accepted, one must further conclude that histamine acts on the same parasympathetic receptive mechanism, for the obvious reason that its action is abolished by large doses of mecholyl. But this conclusion is not acceptable because it is inconsistent with the fact that atropine is unable to abolish histamine secretion (16). This contradiction arises from the first assumption that susceptibility to the action of atropine is a criterion for the intracellular site of action of a drug. If atropine does not afford such a criterion how then can the facts be reconciled? One solution may be reached on the basis of Dale's hypothesis (19) that atropine acts by rendering the cell impermeable to certain drugs. Employing this hypothesis one might say that atropine prevents the access of mecholyl to the intra-cellular receptive mechanism, and thereby abolishes both of its actions, but in the case of histamine, atropine renders the cell only partially impermeable. This explanation, although it eliminates contradictions, does not permit any conclusion as to whether mecholyl exerts its diverse effects on only one intracellular site or two, nor does it permit any conclusion as to whether mecholyl and histamine stimulate the parietal cell at the same or different sites.

CONCLUSIONS

1. Acetyl-β-methylcholine is shown to be very potent in stimulating a flow of acid gastric secretion in dogs.

2. Acetyl-β-methylcholine shows a reversal effect with dosage, large doses stimulating a flow of mucus, and inhibiting the secretion of acid stimulated by histamine.

3. It is shown that both the excitatory and inhibitory effects of acetyl-β-methylcholine can be completely abolished by atropine.

4. The problem of the site of action of the various drugs employed is discussed in the light of the present results.

5. It is shown that the previous contradictory reports in the literature concerning the effect of choline compounds on gastric secretion may be explained on the basis of dosage.

REFERENCES

(1) Dale and Feldberg. J. Physiol. **81:** 320, 1934.
(2) Vineberg. This Journal **96:** 363, 1931.
(3) Suda. Virchow's Arch. **251:** 56, 1924.
(4) Nöll. Arch. f. exp. Path. u. Pharmakol. **167:** 258, 1932.
(5) Masek. C. R. Soc. de Biol. **122:** 430, 1936.
(6) Gavazzani. Clinica med. ital. 1935, quoted by Masek.
(7) Gebhart and Klein. Klin. Wchnschr. **12:** 535, 1933.
(8) Merklen, Warter and Kobaker. C. R. Soc. de Biol. **111:** 1013, 1932.
(9) Wilkinson. Brit. J. Exper. Path. **13:** 141, 1932.
(10) Abbott. Am. J. Med. Sci. **186:** 323, 1933.
(11) Da Costa. Arch. des mal. de l'app. dig. **25:** 921, 1935, quoted by Masek.
(12) Ferguson and Smith. J. Physiol. **83:** 455, 1934-35.
(13) Myerson, Rinkel and Dameshek. New Eng. Med. J. **215:** 1005, 1936.
(14) Simonart. J. Pharmacol. Exper. Therap. **46:** 157, 1932.
(15) Conroe and Starr. J. Pharmacol. Exper. Therap. **49:** 283, 1933.
(16) Gray, Bradley and Ivy. This Journal **118:** 463, 1937.
(17) Feldberg and Vartiainen. J. Physiol. **83:** 103, 1934.
(18) Cannon and Rosenblueth. This Journal **119:** 221, 1937.
(19) Dale and Gaddum. J. Physiol. **70:** 109, 1930.
(20) Gray. This Journal **120:** 657, 1937.

RAPID DECOMPRESSION FOLLOWING INHALATION OF HELIUM-OXYGEN MIXTURES UNDER PRESSURE

EDGAR END

From the Department of Physiology, Marquette University School of Medicine, Milwaukee, Wisconsin

Received for publication June 23, 1937

The problem of determining decompression tables for divers using helium-oxygen mixtures in the newly-developed Craig-Nohl diving suit has resulted in findings of the most encouraging nature. This is the report of a rigorous test of the suitability of helium-oxygen mixtures for use by men working under pressure. As far as I have been able to determine, this is the first paper dealing with such work on human subjects.

The invention of the diving dress, the caisson, and compressed-air tunneling were followed by the observation that on decompressing after exposure to increased pressures, men often suffered from untoward symptoms. Various names such as the bends, caisson disease, divers' palsy, and compressed-air illness were given to these symptoms. In 1878 Paul Bert described bubbles in the vessels of animals rapidly decompressed after exposure to increased pressure, and to these bubbles he attributed the symptoms just mentioned. Bert found by analyzing the gas in these bubbles that they consisted chiefly of nitrogen, the principal inert constituent of air, which he believed had come out of solution in the blood and tissues so rapidly as to form gas emboli.

Bert's work was followed by little advancement in the prevention of compressed-air illness until 1908 when Boycott, Damant and Haldane published tables establishing the principles of stage decompression. Recent work by Hawkins, Shilling and Hansen (1935) has suggested changes in favor of shorter decompression. Otherwise, little has been done to permit a diver to descend to great depths or to remain under pressure for long periods of time without spending an exorbitant amount of time in decompressing. It is largely this length of time spent in decompressing which limits productive diving to relatively shallow water. For example, according to the decompression tables used by the United States Navy (1925), a diver who descends to a depth of two hundred and fifty feet and remains there for twenty minutes must spend two hours and twenty-six minutes in being drawn back to the surface.

Some years ago Dr. J. H. Hildebrand, of the University of California, suggested that if inert nitrogen is the cause of compressed-air illness,

substitution of a less soluble gas for nitrogen in the air breathed by men under pressure would permit more rapid decompression. Helium, which was at that time becoming available in substantial quantities, presented the ideal characteristics of being inert, odorless, tasteless, colorless, less soluble than nitrogen, and also more diffusible than nitrogen. Working with Sayers and Yant (1925), Hildebrand found that when animals inhaled helium-oxygen mixtures under increased pressures they could be decompressed safely in one-fourth to one-third of the time required for safe decompression when nitrogen-oxygen mixtures were breathed.

To use helium-oxygen mixtures for ventilating the standard type of diving dress which receives its air supply from the surface is impractical. Recently the Craig-Nohl diving dress, a self-contained unit designed to utilize the findings of Sayers, Yant, and Hildebrand regarding helium-oxygen mixtures, has been developed for salvage work on the Lusitania. The physiological problems involved in developing this equipment have been worked out in this laboratory. A helium-oxygen mixture is used to inflate the Craig-Nohl suit as the diver descends, and when the working level has been reached flow of this mixture is stopped and oxygen is introduced at a carefully controlled rate to satisfy the diver's metabolic requirements. With this equipment the diver is independent of the surface for his air supply, carrying his helium-oxygen mixture and additional oxygen in cylinders on his back.

In order to obtain data for determining decompression tables for divers breathing helium-oxygen mixtures, Captain John D. Craig and Mr. Max E. Nohl, designer of the Craig-Nohl diving dress, have served as voluntary subjects for tests which appear to establish beyond question the superiority of this artificial gas mixture over ordinary air for men working under pressure. Mr. Nohl is twenty-six years of age, and Captain Craig is thirty-three. Both of these men are experienced divers capable of making critical comparisons between the new mixtures and compressed air. The experiments reported in this paper were performed in a steel recompression chamber in the Milwaukee County Emergency Hospital through the courtesy of its designer, Mr. Joseph C. Fischer, Chief Engineer of the Milwaukee County Institutions. This recompression chamber is eighteen feet long and seven feet in diameter and is divided into an inner and an outer compartment by pressure-tight doors. In it pressures up to forty-four pounds per square inch are available.

For these experiments two sets of rebreathing apparatus were constructed, each consisting of a spirometer, a soda-lime chamber, valves, an air-tight mouthpiece, and necessary tubing and connections. Helium-oxygen or helium-nitrogen-oxygen mixtures prepared beforehand were admitted to these systems from a cylinder under pressure, and oxygen was added from another cylinder at will. The subjects breathed from

these systems during the entire period of each experiment. The gas mixture in the systems was frequently changed to insure accuracy, and addition of oxygen to replace that consumed by the subject was carefully controlled.

The work of Sayers, Yant, and Hildebrand showing that small animals could be decompressed three or four times as rapidly from helium-oxygen mixtures as from nitrogen-oxygen mixtures formed the basis for these experiments. In addition, consideration was paid to the fact that helium is less soluble than nitrogen, is more rapidly diffusible, and dissolves in drawn blood in direct proportion to the helium pressure according to Henry's law (Hawkins and Shilling, 1936). Hildebrand, moreover, (personal communication) believes that the ratio of the solubility of helium to nitrogen is much less in lipoids than in water, which is an extremely favorable factor inasmuch as the great lipoid solubility of nitrogen makes every lipoid tissue in the body a dangerous storage depot for this gas under pressure.

Two methods of experimentation presented themselves for determining a decompression schedule for helium-oxygen mixtures. In one method, a pure helium-oxygen mixture could be breathed for a fixed period of time in a series of experiments in which the pressure increased with each succeeding experiment. In the other method, time and pressure could be kept constant, and the partial pressure of helium could be increased with each experiment. This latter method was chosen because of the convenience with which it can be employed during actual diving operations. The partial pressure of helium can be increased conveniently by using it to dilute and finally replace the nitrogen in prepared mixtures. In these experiments, by maintaining the concentration of oxygen at twenty-one per cent, easy comparison with atmospheric air was possible.

For the purpose of these experiments a graph was made (fig. 1) with pressure in equivalent feet of sea water as ordinates and time as abscissae. Decompression schedules for divers working in ninety feet, sixty feet, and thirty feet of water are shown. These represent, respectively, the nitrogen decompression schedules for three-thirds, two-thirds, and one-third of the maximum depth (ninety feet) or for gas mixtures in which nitrogen makes up three-thirds, two-thirds, or one-third of the total inert gas being breathed. An assumed helium decompression line for ninety feet was drawn as an unbroken line indicating decompression from maximum pressure to atmospheric pressure at a uniform rate and in one-fourth of the time necessary for nitrogen. The work of Sayers, Yant, and Hildebrand showed that animals could be decompressed safely from helium-oxygen mixtures in one-fourth of the time necessary when nitrogen-oxygen mixtures were breathed. Because of the lower solubility of helium and its more rapid rate of diffusion, decompression from helium-

oxygen mixtures at a fairly uniform rate appears to be preferable to stage decompression.

In diving work the rate of compression (i.e., descent) is limited in practice only by the ability of the diver's ears to adjust themselves to changes in pressure and ordinarily occurs at the rate of forty feet a minute. As will be appreciated by referring to figures 2, 3, and 4, however, the length of time necessary to build up pressures to a maximum in the recompression chamber used in these experiments is enough to alter calculations considerably. This factor had been disregarded in previous work until a subject suffered from painful and disabling cramps in both arms following a decompression which took into consideration only the time spent at maximum pressure. Following this unfortunate experience, two-thirds of the time spent in compressing from atmospheric pressure to maximum pressure were added to the time spent at full pressure when computing decompression schedules. The value two-thirds was arbitrarily selected in preference to the obvious one-half because of the decreasing rate of pressure rise as maximum pressure was approached (not indicated in figs. 2, 3, and 4) due to air compressor characteristics.

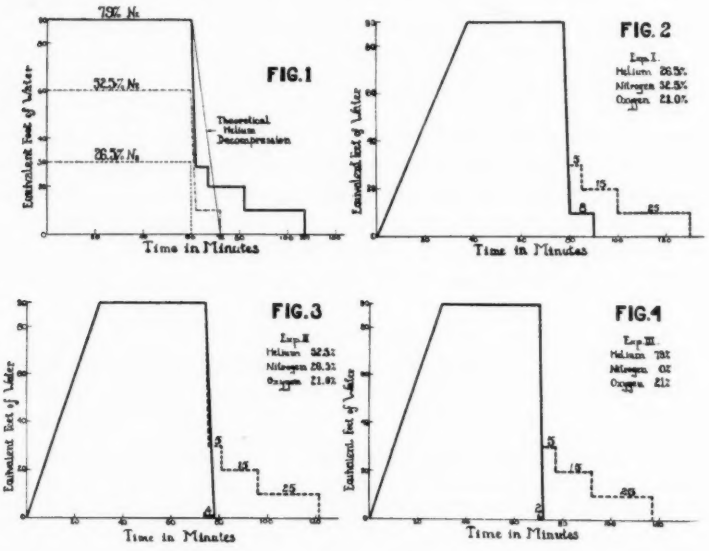
According to the helium content of the mixtures breathed, this experiment can be divided into three parts. The following mixtures were calculated and mixed to provide, respectively, a helium content equal to one-third, two-thirds, and finally three-thirds of the inert gas in the mixture.

	O_2	N_2	He
Experiment I	21 per cent	52.5 per cent	26.5 per cent
Experiment II	21 per cent	26.5 per cent	52.5 per cent
Experiment III	21 per cent	0 per cent	79 per cent

Inasmuch as the writer alone breathed air throughout the experiments while making observations and adjusting apparatus, it was necessary to employ the innermost room of the decompression chamber for decompressing him at a slower rate while he observed the decompression of the helium-breathing subjects through a small window.

By referring to figure 1 it will be seen that in the first experiment, in which nitrogen made up two-thirds of the inert gas in the mixture being breathed, the subjects were under the same conditions as a diver breathing air at a depth of sixty feet, as far as the nitrogen content of their bodies was concerned. Decompression according to standard tables after exposure at sixty feet for one hour's time requires twelve minutes. Actually (fig. 2) only eight minutes were spent in decompressing the two subjects. There were two reasons for this reduction in decompression time. The first reason was that while the standard decompression tables incorporate a substantial margin of safety, these experiments were an attempt to reduce decompression time to a minimum. The second reason was that

there was actually less nitrogen in the body than was calculated, due to the fact that the subjects breathed a mixture relatively deficient in nitrogen, and early during the period of compression there was actual loss of nitrogen from the body to establish equilibrium with the gas in the rebreathing apparatus, this lost nitrogen being disposed of each time that the apparatus was purged and refilled with a fresh mixture. Decompression in eight minutes' time in the first experiment was followed by no bad effects. This result justified the reduction made and provided a basic figure from which to work with helium. In the two succeeding experiments the nitrogen content was too low to require consideration in the computation of decompression schedules (fig. 1).



In the second experiment, with the subjects breathing a mixture in which helium made up two-thirds of the inert gas, it was decided to halve the decompression time of the preceding experiment in order to bring out any deleterious effects that helium might be capable of producing. It will be remembered that the partial pressure of nitrogen in this experiment was too low to require consideration in computing a decompression schedule. As is depicted in figure 3, uneventful decompression in four minutes' time was made after approximately one hour's exposure to a pressure equivalent to ninety feet of sea water. Decompression at a uniform rate rather than by steps or stages was employed.

In the final experiment of the series (fig. 4), a mixture of 79 per cent

helium and 21 per cent oxygen was breathed by the two subjects under a pressure equivalent to ninety feet of sea water for a period of approximately one hour. At the end of that time, by employing several emergency valves in addition to the regular outlets on the recompression chamber, the two subjects were decompressed in two minutes without any apparent ill effects. The reduction in decompression time, as computed against standard navy tables, is forty-five minutes. The ratio of helium-decompression to nitrogen decompression in this instance is as 1 is to 23.5. The temperature in the chamber fell from 72°F. to 45°F. during the period of decompression.

Aside from raising the pitch and altering the quality of the voice, inhalation of helium-oxygen mixtures under pressure appeared to have no unusual effect. It is my belief that in addition to shortening the time necessary for safe decompression, helium may also free divers from the untoward psychological effects of air at high pressures. Since Behnke, Thomson, and Motley (1935) called attention to the fact that the lipoid coefficient of nitrogen is great enough to cause it to be suspected as the cause of the "narcotic" effect of air at high pressures, experiments in this laboratory on the effects of helium on the state of consciousness of men working under pressure have been very encouraging. The relative insolubility of helium in lipoid tissue makes it an ideal gas for use in testing the theory of Behnke, Thomson, and Motley.

In this paper no attempt has been made to discuss the cause of compressed-air illness or the factors influencing susceptibility to it. Consideration of these problems in the light of complicating phenomena observed in this laboratory will be attempted in a later paper. In this connection, however, it is interesting to note that shortening of the coagulation time of the blood after decompression has been found consistently.

While the results reported in this paper serve fully to justify the writer's expectations for helium-oxygen mixtures, they are reported in the hope that they will stimulate more work by others in this very promising field. Only by careful investigation such as is being carried on in developing decompression tables and other data for use with helium-oxygen mixtures in the Craig-Nohl diving dress will helium-oxygen mixtures be made universally available to those individuals who are subjected to the dangers of compressed-air illness. Above all, the fact that two persons have suffered no apparent ill effects following decompression in two minutes' time after having breathed a helium-oxygen mixture at a pressure equivalent to ninety feet of sea water for approximately one hour should not be interpreted by anyone as an attempt to establish this as a safe rate of decompression under the circumstances. Such figures are, however, highly encouraging.

Grateful acknowledgment is made to Mr. James T. Howington, Vice

President of the Girdler Corporation, for the helium used in these experiments.

SUMMARY

1. After breathing a helium-oxygen mixture under pressure, two subjects have been uneventfully decompressed in less than one twenty-third of the time required when compressed air is breathed.

2. Attention is called to several possibilities of helium-oxygen mixtures as a substitute for compressed air for men working under increased pressures.

REFERENCES

BEHNKE, A. R., R. M. THOMSON AND E. P. MOTLEY. This Journal **112**: 554, 1935.

BERT, P. La Pression Barometrique. Paris, 1878.

BOYCOTT, A. E., G. C. C. DAMANT AND J. S. HALDANE. J. Hyg. **8**: 342, 1908.

HAWKINS, J. A., C. W. SHILLING AND R. A. HANSEN. U. S. Nav. Med. Bull. **33**: 327, 1935.

HAWKINS, J. A. AND C. W. SHILLING. J. Biol. Chem. **113**: 649, 1935.

SAYERS, R. R., W. P. YANT AND J. H. HILDEBRAND. Report of Investigations—Bureau of Mines. R. I. no. 2670, 1925.

United States Navy Department Diving Manual. Washington, 1925.

ELECTROLYTE AND WATER CHANGES IN MUSCLE DURING ATROPHY

H. M. HINES AND G. C. KNOWLTON

From the Department of Physiology, State University of Iowa

Received for publication June 24, 1937

This investigation was undertaken in order to determine the nature and extent of changes in the electrolyte and water content of skeletal muscle undergoing various degrees and types of atrophy. Additional information is presented concerning the distribution of electrolytes and water between the muscle cells and interstitial phases of normal tissue.

The studies were carried out on the gastrocnemius muscles of approximately 150 adult rats. In all experiments, except those concerned with fasting, the homologous muscle from the unoperated limb served as a control and was considered as normal muscle. The various experimental types of atrophy investigated were those associated with fasting, denervation, tenotomy and fasting concomitant with denervation. The muscle was tenotomized by chipping off the os calcis and sewing this attachment to the sutured skin. Because of regeneration tendencies of the tendon such studies were limited to a period of two weeks. Denervation was accomplished by removing a section of the sciatic nerve about 1 cm. in length. In the fasting experiments the sciatic nerve was sectioned and the animals deprived of all food except water for a period of 8 days during which the average decrease in body weight was 29 per cent. The animals were killed by hemorrhage and the removal of blood from the tissues was aided by limb vein section and massage. The muscles were weighed after being dissected free, as far as possible, from tendon and superficial fascia. Their water content was determined by drying to a constant weight in an electric oven at 106°C. The determinations of chlorides were made by the method of Sunderman and Williams (1933). For the potassium determinations the dried muscles from ten animals in each series were pooled, ashed and analyzed according to Shohl and Bennett (1928). A suitable number of muscles were pooled, dry ashed and analyzed for calcium according to the technique described by Taubmann (1934). Perfusion experiments were carried out on 5 rats, 9 days after a unilateral section of the sciatic nerve had been made. A cannula was inserted into the lower abdominal aorta. The leg veins were severed and the hind limbs were perfused for 30 minutes with 225 cc. of Ringer's solution by means of a Woodyatt pump. The

animal and injection fluid were kept at a temperature of 37°C. Chloride and water determinations were made on the control and denervated gastrocnemius muscles.

The data (table 1) show that muscular atrophies caused by denervation, fasting and tenotomy were associated with an increased chloride concentration in the tissue which, in degree, paralleled the extent of atrophy. It is apparent from the studies of numerous investigators that the normal muscle cell is free from and impermeable to chloride. The chloride content of skeletal muscle is usually attributed to that contained in the fluid of the intercellular spaces. It is not clear as to what portion of the muscle chloride might be contained within the less specialized cells such as the various types of connective tissue. The observation of Manery (1937) that the

TABLE 1

Average values for the changes in electrolyte and water content of gastrocnemius muscle during atrophy

Millimols or cubic centimeters per 100 grams dry weight

CONDITION	PER CENT OF WEIGHT LOSS	WATER	CALCIUM	CHLORIDE	POTASSIUM	ACID-SOLUBLE PHOSPHORUS
		cc.	*mM*	*mM*	*mM*	*mM*
Denervation						
3 days	6.7	+1.22		+1.443	−1.17	
7 days	28.2	+0.69	+0.086	+2.326	−3.37	−0.85
14 days	49.9	+1.87	+0.169	+5.756	−2.99	−2.99
28 days	72.3	+2.53	+0.743	+10.603	−13.76	−7.73
Tenotomy						
10 days	27.9	+2.15	+0.185	+2.985		
Fasting						
8 days	26.0	+2.72	+0.128	+2.323		

per cent of chloride in muscle is lowered by removal of its tendon and accessible fascia suggests that it may be erroneous to assume that muscle chlorides are associated exclusively with extracellular fluid. It has been observed that the dominant changes in rat's skeletal muscle during the first few weeks following denervation are those brought about by a loss of muscle cell substance and a relative increase in the non-muscle cell phases (Hines and Knowlton, 1933). Calculations have been made (table 2) of the amounts of chloride per unit of tissue weight that should be present in the various atrophic muscles if the material lost was a chloride free muscle cell substance. The values obtained from the analysis of the control muscle in the opposite limb served as data for the calculations of values for denervation and tenotomy. The original weights of the muscles in the fasting experiments were calculated from the body and muscle

weight ratios as described by Hines and Knowlton (1934). The agreements between the calculated and the actual values found by analysis were very close in most instances. The increase in chloride concentration was parallel to the relative increase in connective tissue.

The data (table 1) show that all degrees and types of atrophy were accompanied by slight but consistent increases in the total water content of muscle. The distribution of water between the muscle cell and non-muscle cell phases has been estimated (table 2) from the data on rat muscle

TABLE 2

Average values for electrolyte and water distribution in normal and atrophic muscle
(Millimols or grams per 100 grams of wet tissue)

	CHLORIDE		MUSCLE CELL WATER	NON-MUSCLE CELL WATER	NON-MUSCLE CELL PHASE WHEN THIS PHASE IS		WATER IN MUSCLE CELL PHASE WHEN NON-MUSCLE PHASE IS		MILLIMOLS PER 100 GRAMS MUSCLE CELL WATER		MILLIMOLS CALCIUM PER 100 GRAMS NON-MUSCLE CELL PHASE
	Found	Calculated			99 per cent water	80 per cent water	99 per cent water	80 per cent water	K	P	
	mM	*mM*									
Normal	1.34	1.34	65.0	10.8	10.9	13.5	73.0	75.1	14.5	7.8	0.9
Denervated											
3 days	1.71	1.61	62.7	13.8	14.0	17.3	72.9	75.8	14.6		
7 days	1.95	1.81	60.4	15.8	16.0	19.7	71.9	75.2	13.1	8.2	0.7
14 days	2.62	2.56	55.6	21.2	21.4	26.5	70.7	75.6	14.0	7.5	0.7
28 days	3.68	4.46	47.4	29.8	30.1	37.3	67.8	75.6	15.1	6.5	0.8
Tenotomy	1.96	1.80	61.1	15.9	16.0	19.8	72.7	76.2			0.8
Fasting											
Control	1.80	1.78	62.5	14.6	14.7	18.3	73.3	76.4			0.8
Denervated	2.44	2.30	57.5	19.8	20.0	24.8	71.9	76.5			
Perfused											
Control	5.69		45.5	36.0		38.7*		74.2			
Denervated	6.38		41.7	40.4		45.0*		75.9			

* Equals non-muscle cell water plus amount of solids found in unperfused control muscle.

and serum and the equations as described by Hastings and Eichelberger (1937). The results show that all types of experimental atrophy exhibit a decreased amount of muscle cell and an increased amount of non-muscle cell water. These changes in water distribution paralleled the decrease in muscle cell size and the relative increase in connective tissue as revealed by microscopic studies. The assumption that all of the chlorides are present in intercellular spaces as a filtrate from plasma, requires that as much as 30 per cent of the weight of a muscle at the end of a 4 weeks' period of denervation be represented by extracellular fluid. The appear-

ance and properties of such muscles are not in accord with such an assumption. It is believed, therefore, that the above observations are evidence that a large amount of the muscle chlorides is associated with the connective tissue cells.

The concentration of water in the muscle cell phase has been calculated in two ways. The first was according to the formulae of Hastings and Eichelberger where the amount of intercellular phase equals the total minus the extracellular phase. In this calculation the concentration of water in the extracellular phase approximates that of an ultra filtrate from serum. The second method of calculation assumes that the chlorides are chiefly associated with connective tissue having a water content of 80 per cent. It is to be noted that this value is an approximation only. It was found that by the first method of calculation the per cent of muscle cell water progressively decreases as atrophy ensues; while the second method of calculation allows for approximately a constant water concentration in the normal and atrophic muscle cells.

The data (table 1) show that the concentration of acid-soluble phosphorus and potassium in the atrophic muscles was decreased by amounts which paralleled the degrees of atrophy. It is recognized that these substances are chiefly associated with the contents of the muscle cell rather than the interstitial phases. Calculations have been made (table 2) of the concentration of acid-soluble phosphorus and potassium in muscle cell water, after deducting from the total the estimated amounts that would be included in the non-muscle cell phases if present in concentrations governed by ultrafiltration. It is apparent that muscle cells undergoing atrophy of denervation have essentially a normal concentration of acid-soluble phosphorus and potassium in their cell water.

The data (table 1) show increased amounts of calcium in the atrophic muscles. This increase, however, appears to be correlated with a relative increase in the amounts of interstitial tissue. This is more apparent when the calcium values are expressed (table 2) in concentration per 100 grams of non-muscle cell phase. The observed values for muscle calcium are too great to permit it to be considered as an ultra filtrate from serum. This suggests that some of the muscle calcium may exist in combination with tissue substances. The paucity of information concerning the state of calcium in muscle prohibits further analysis of these data.

The question might be raised as to whether the atrophic muscle cells exhibit some degree of permeability to chlorides. Such a condition is regarded as unlikely because of the appearance and properties of such cells and the fact that three widely different experimental procedures leading to atrophy, such as denervation, fasting and tendon section, all result in essentially the same change in muscle chloride for equivalent weight losses. The results of the perfusion experiments may, likewise, be cited

as evidence that the atrophic cells possess a normal state of impermeability to chloride. The amounts of chloride found in both the perfused control and denervated muscles are such as can be attributed to the non-muscle cell phases of these tissues.

The assumption that the chloride containing phases of muscle tissue are chiefly associated with connective tissue rather than extracellular fluid necessitates a revision of the usual formulae for estimating the values for muscle cell and non-muscle cell phases and the concentration of water in the same. However, when estimations are made on the basis that the non-muscle cell phase contains 80 per cent water it is apparent that muscle cells may undergo considerable reduction in volume due to atrophy from different causes and maintain approximately a normal electrolyte and water content. It is believed that many of the findings of altered concentration of various substances in atrophic muscles can be attributed to relative changes in the amounts of muscle cell and non-muscle cell phases rather than to a change in the composition of the various phases.

SUMMARY

Studies were made concerning the electrolyte and water content of normal and atrophic gastrocnemius muscles of the rat. The atrophies were produced by denervation, fasting and tenotomy.

The muscles undergoing the various types of atrophy exhibited a slight increase in total water concentration. The increase in chloride concentration of atrophic muscle was found to be parallel to the extent of atrophy and the relative increase in connective tissue. This finding is regarded as evidence that the chlorides of muscle are chiefly associated with the connective tissue rather than the extracellular spaces.

Estimations have been made of the distribution of water between the muscle cell and non-muscle cell phases of such tissues. Such calculated values indicate that atrophic muscle cells have a normal concentration of acid-soluble phosphorus, potassium and water. The increase of calcium in muscle during atrophy was parallel to the increase in connective tissue.

REFERENCES

HASTINGS, A. B. AND L. EICHELBERGER. J. Biol. Chem. **117:** 73, 1937.

HINES, H. M. AND G. C. KNOWLTON. This Journal **104:** 379, 1933. This Journal **110:** 8, 1934.

MANERY, J. F. This Journal **119:** 372, 1937.

SHOHL, A. T. AND H. B. BENNETT. J. Biol. Chem. **78:** 643, 1928.

SUNDERMAN, F. W. AND P. WILLIAMS. J. Biol. Chem. **102:** 279, 1933.

TAUBMANN, G. Arch. f. Exper. Path. u. Pharmakol. **174:** 476, 1934.

ELECTRICAL STUDIES OF OVULATION IN THE RABBIT

J. REBOUL,[1] H. DAVIS AND H. B. FRIEDGOOD[2]

From the Department of Physiology, Harvard Medical School, Boston

Received for publication June 25, 1937

It is well recognized that the rabbit usually ovulates only after mating and that the ovarian follicles rupture within 10 to 12 hours post coitum. In 1935 Burr, Hill, and Allen of Yale reported that ovulation in the rabbit is associated with a characteristic electrical change. In a previous communication, Reboul, Friedgood and Davis (1937) reported confirmation of their results. The phenomenon is a slow potential which can be conveniently detected between the vagina and the abdominal wall, provided appropriate instruments are employed. It is essential in such experiments to use a circuit which is sensitive to changes of potential but which is independent of changes of resistance in the input circuit. It is necessary, furthermore, to employ non-polarizable electrodes. In conformity with these principles, Burr, Lane and Nims (1936) developed silver–silver chloride electrodes and a sensitive vacuum-tube microvoltmeter possessing a high degree of stability. A further study of the nature of the ovulation potential was undertaken on the basis of these technical data. Electrodes were prepared according to their specifications, and have proved extremely satisfactory. A vacuum-tube voltmeter was built according to their wiring diagram and specifications, and many of the present experiments were carried out with it. Its stability was adequate for our purposes, although we never attained quite the degree of steadiness which Burr and his associates describe. Its drift, however, was not greater than 100 microvolts per hour and often much less. Subsequently, one of us (J. R.) with the collaboration of Mr. Albert Grass devised a somewhat simpler vacuum-tube circuit which employs only one tube, and proved to be quite as steady as, although slightly less sensitive than the earlier instrument (see fig. 1). The vacuum-tube circuit operates a D'Arsonval mirror galvanometer so adjusted, with the new circuit, as to give a 5 mm. deflection for 1 millivolt potential difference applied to the input leads of the amplifier, and a natural period of 8 seconds. A shunt resistance, which was sufficient to damp the instrument critically, was employed.

[1] Victor Emmanuel Chapman Memorial Fellow, Harvard University.

[2] Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

METHOD. The female rabbits used in these experiments were anesthetized with intravenous urethane (0.8 gram per kilo) about 7 to 9 hours after mating. A wick, connected to one electrode by rubber tubing which was filled with salt solution, was placed in the vagina. The

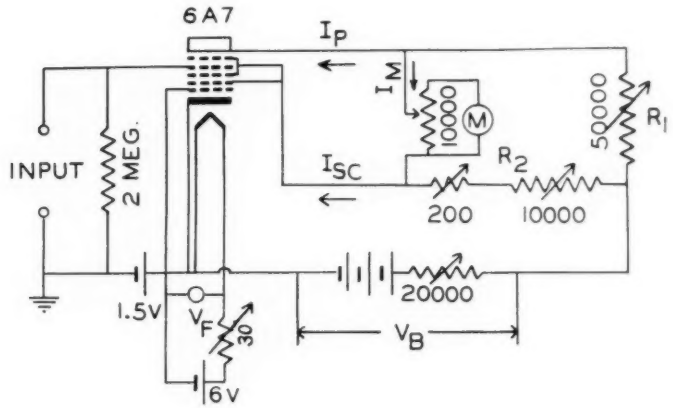


Fig. 1. This circuit is comparatively simple, inexpensive, and yet has the required sensitivity and stability. Since both I_p and I_{sc} change in the same way with a change in V_f, the difference, I_m, remains fairly constant over small ranges, while a change in the input grid voltage changes only I_p. The operating point on the curve $I_m = f(V_f)$ is chosen where the slope $\frac{d\ I_m}{d\ V_f}$ is a minimum. The condition for minimum effect due to drift in the plate battery is $\frac{R_2}{R_1} = \frac{r_p}{r_{sc}}$ where r_p and r_{sc} are the internal plate to cathode and screen to cathode resistances respectively. This means that the resistance bridge which makes up the plate-screen circuit must be balanced, i.e., I_m must be zero, a condition very easily fulfilled. It is necessary to fulfill both conditions simultaneously for minimum drift, but this can always be done with the specified circuit constants. For a particular tube, we found the desirable heater voltage to be 3 and the plate voltage to be 12.

The sensitivity is such that an input signal of one millivolt gives 7×10^{-7} amperes in the galvanometer (M).

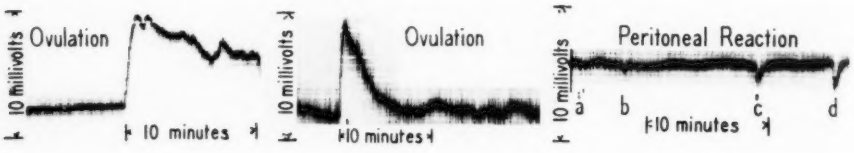
An ordinary milliammeter may be placed in the galvanometer circuit to facilitate rapid setting of approximate balance of the resistance bridge of the plate-screen circuit.

Numerals in the figure give the values of the resistance in ohms.

abdominal wall was shaved, and another wick electrode applied to the supra-pubic region. Occasionally a small opening was cut in the skin, and the wick was placed in contact with the underlying fascia or muscles. The potential difference during the period of ovulation was read from time to time on a scale or recorded photographically on bromide paper. The

abdomen was subsequently opened and the number of ruptured follicles counted. More commonly the abdomen was opened before ovulation began; and the ovaries, still in their normal position, were observed during the recording. The instant of rupture of a follicle was usually signalled by a beam of light on the photographic paper.

RESULTS. It is somewhat difficult to determine the exact instant of rupture even by close inspection; and, because of the slow period of the galvanometer, the exact beginning of the electrical deflection is also uncertain within 1 or 2 seconds. With one observer watching the ovary and the other the galvanometer, we determined, however, that the potential change did not precede the rupture, but seemed to follow it within 5 to 15 seconds.



A B C

Fig. 2. Photographic records of the electrical potential between vagina and abdominal wall of an anesthetized ovulating rabbit. Upward deflection indicates vagina more positive. The vertical bands in the record are due to discontinuous movement of the bromide paper. Each band corresponds to a 15 second interval.

A. Typical ovulation potential, abdomen unopened. "Slow" return to the baseline.

B. Peritoneal cavity open, ovary *in situ*, ovulation directly observed. "Quick" return to baseline.

C. "Peritoneal reaction." Abdominal cavity open.

a. Normal Ringer's solution dropped into peritoneal cavity.

b. Intestine touched with glass pipette.

c. Solution of 2 per cent NaCl and 1 per cent KCl dropped into peritoneal cavity.

d. Peritoneal cavity dried with cotton.

Our results, for the most part, confirm those of Burr, Hill and Allen. A potential difference appears between vagina and abdominal wall in association with ovulation. The vagina becomes more positive during this period. The potentials recorded by our apparatus were smaller than those reported by Burr et al. Usually the potentials ranged from 6 to 10 mv., with the maximum at 12 mv. and the minimum at 5 mv. The initial potential change is so rapid that the movement of the spot is apparently determined by the characteristics of our galvanometer. The return to the baseline, however, is slow. In many cases this requires 25 minutes, although occasionally there is a quicker return requiring only 5 to 10 minutes. The return to the baseline is asymptotic along an approximately exponential curve (fig. 2, A and B).

Burr and his associates also mention still slower fluctuations in the resting potential in the period during which ovulation occurs. We have not observed such changes. In fact, our baseline has been steady consistently within 1 mv. except during manipulations of the animal or electrodes.

The abrupt deflection with slow return was invariably associated with the rupture of an ovarian follicle; and a change of similar polarity, magnitude, and time-course was not observed in the absence of ovulation. One or two obvious artefacts might cause somewhat similar changes, viz., breaking the continuity of the fluid-column in the tubing connecting the animal to the electrodes, or urination. When urine comes in contact with the vaginal electrode, a large quick deflection always occurs, but the change of potential is essentially permanent and does not return to the original zero. Because of the slow period of our galvanometer, muscular contractions did not cause a significant deflection as long as good contact of the electrodes was maintained. We found it possible also to manipulate the animal, i.e., open the abdomen, without seriously disturbing the electrical record. In all experiments, the animal-board and the operator were carefully insulated from ground, the operator wore rubber gloves, and the use of bimetallic instruments was avoided. Brief deflections due to static charges were encountered occasionally.

After we had thus confirmed the existence of the ovulation potential, we undertook to determine its source. We first tested the possibility that it might be due to the emptying of the ovarian follicle. A fine glass pipette was therefore inserted into the ripe follicle and the fluid carefully withdrawn. This entire procedure caused only a small quick deflection of less than 1 mv., opposite in direction to the ovulation potential. Apparently, the emptying of the follicle is not the critical event. The contents of the pipette were then dropped into the abdominal cavity. A small quick deflection of less than 1 mv., opposite in direction to the ovulation potential, appeared once more. We term this type of deflection the "peritoneal reaction" (fig. 2, C). It can be produced by dropping into the abdominal cavity other fluids, such as 0.9 per cent sodium chloride, warm-blooded Ringer's solution, cold-blooded Ringer's solution with excess of sodium chloride or potassium chloride. The same peritoneal reaction was observed following such mechanical trauma as touching or scraping the peritoneum with a glass pipette or wiping it with dry cotton. The change in potential was smaller or absent when similar procedures were applied to exposed muscles or fascia. It also appeared when the ovary was manipulated with gloved fingers. It is clearly distinct from the ovulation potential, but we have not yet determined its exact nature or the locus of its production. In all of the procedures described above, one electrode was in the vagina, the other in contact with the peritoneum or the abdominal musculature or fascia.

A number of observations were made on ovarian follicles which were ruptured manually. In the early experiments, the ovary was lifted in gloved fingers and pressure was applied to the base of the follicle. This procedure always produced blood as well as follicular fluid from the ruptured follicle, and also involved lifting the ovary out of its normal position. Under these conditions, rupture did not always yield an ovulation potential, but we observed deflections somewhat resembling the ovulation potential when the ovary was replaced in its normal position shortly after a rupture. This type of experiment was soon abandoned because of the possibilities of internal damage to the ovary and significant alterations of its electrical contacts.

In another series of observations, rupture was induced by inserting a pipette into the substance of the ovary and injecting either salt solution or air at the base of a follicle until the increase of internal pressure induced its rupture. In one experiment, two ruptures were associated with characteristic ovulation potentials, while one was not. In another experiment, one rupture gave the potential, while a second rupture failed to do so. We believe that the positive results are more significant than the negative, since failure to show a potential might be due to an undeveloped or atretic condition of the follicle.

The origin of the ovulation potential was finally localized in the ovary. The ovary was externalized through a small opening in the skin and abdominal wall, and separated from contact with the other tissues by packing with dry cotton. The only remaining contact was through the blood vessels and the Fallopian tube attached to one end of the ovary. In several experiments, no ovulation occurred under these conditions. Failure of ovulation may have been due to lowering of the temperature of the ovary or, more probably, to partial interference with the blood supply. In three cases, however, ovulation did occur, but these ruptures were not associated with ovulation potentials.

In a final series of experiments we inserted a micro-pipette electrode (about 0.1 mm. diameter) into ovary or follicle, or brought it in contact with the external surface of the ovary or of a stimulated preovulatory follicle. The reference electrode was always in the vagina. These manoeuvers disclosed that the potential over the follicle is more negative than the remainder of the surface of the ovary (fig. 3). The ovulation potential was reproduced by simply inserting the pipette into the substance of the ovary during the intervals between follicular rupture (fig. 4). When the pipette was placed first in contact with the surface of the ovary and then forced into its interior, the potential swung in the negative direction, showing that the interior of the ovary was negative with respect to its surface. After the initial negative deflection which followed the insertion of the electrode into the ovary, the potential slowly

returned to the baseline which had been established while the electrode was in contact with the external surface. The time-course of this return corresponds closely with that of a normal ovulation potential. Both the fast (5 to 10 minutes) and the slow (25 to 30 minutes) types of curve have been obtained.

Attempts were also made to insert the micro-pipette into the interior of a ripe follicle. On account of the toughness of the peritoneal covering,

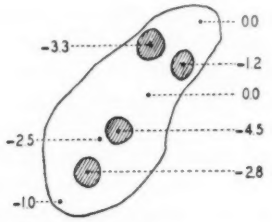


Fig. 3. Distribution of potential (in millivolts) on the surface of a rabbit's ovary with four ripe follicles (shaded). The potential was measured between the vagina and each of the points indicated and the potential of the upper right-hand point arbitrarily selected as zero. Note that all of the follicles are more negative than all of the other points with the exception of the point between the two lower follicles. This point, however, is very close to the most negative follicle.

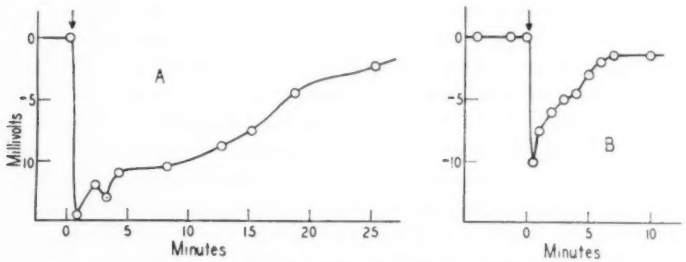


Fig. 4. Potential recorded from within a stimulated ovary following insertion of a micro-pipette electrode. Reference electrode is in the vagina. The potential of the pipette electrode swings sharply negative as the latter pierces the peritoneal covering of the ovary (at arrow), and then returns slowly to nearly its original value. A shows slow return, B the quicker return. (Cf. fig. 2, A and B.)

it proved impossible to do this without at the same time piercing the far side of the follicle and entering the substance of the ovary. This gave a deflection similar to entering the ovarian substance without piercing a follicle. Withdrawal of the electrode until its tip lay within the follicle revealed a potential intermediate between the interior of the ovary and its exterior, when follicular fluid did not escape during this manoeuver.

Micro-pipette electrodes were also inserted into other tissues such as the abdominal musculature, the intestine, and the kidney. No potential differences greater than 1 mv. were detected unless blood or tissue-fluids were sucked into the pipette during the manoeuver. In this case, random variable potentials were obtained, usually much less than those associated with insertion into a stimulated estrous ovary. The ovaries of estrous animals which had not been mated were also tested. The average potential difference was only 1.2 mv. The potential between the inside and outside of an ovulating ovary falls very considerably after interference with its blood supply. In one experiment, three successive ovarian punctures revealed potentials of 6.0 mv., 3.0 mv. and 4.2 mv. while the blood supply was intact; after ligation of the ovarian vessels, another puncture showed a potential of 1.6 mv. In a second experiment, the potentials were 7.0 mv. and 3.0 mv. before ligation of the ovarian vessels and 1.0 mv. after their ligation. A third series of observations revealed potentials of 2.0 mv. and 5.0 mv. before ligation and less than 1.0 mv. after interference with the circulation. The potential difference drops practically to zero with the death of the animal. In one experiment, an ovary was tested three to four hours after mating, but the potentials were no larger than those from a resting ovary, i.e., 1 to 2 millivolts.

Discussion. These experiments establish a close association between the spontaneous rupture of a ripe ovarian follicle and the appearance of a potential between the vagina and various points on the abdominal wall. This potential shows a characteristic polarity, magnitude, and time-course. It does not appear when the ovary is placed in such a position that the usual electrical contacts between the ovary (particularly the rupturing follicle) and surrounding tissues are broken. The electrical phenomenon has been reproduced by inserting a fine capillary electrode into the interior of an ovulating ovary; but a similar potential has not been obtained from other abdominal organs. The polarity of the ovulation potential indicates that it is due to the establishment of electrical contact between the electro-negative interior of the ovary and the surrounding tissues, making the latter more negative with respect to the vagina. This, we believe, is the essential basis of the ovulation potential.

The question then arises whether the ovulation potential may be considered as merely a special case of an injury potential. The rupture of the peritoneal lining covering the follicle is certainly an injury, and the polarity of the observed potential is consistent with this interpretation. This simple view does not cover all of the facts, however, since a large potential is found previous to rupture in the interior of an ovulating ovary, but not in an unstimulated ovary or other tissues, such as muscle or intestine. This pre-existing potential, characteristic of an ovulating ovary, is probably the ultimate source of the ovulation potential, rather than the

fact of injury itself. The rupture of the follicle establishes a new pathway of relatively low electrical conductivity and allows this potential to be manifested externally.

The gradual subsidence of the potential which follows ovulation may be tentatively attributed to processes of recovery or repair following the rupture. The injury potential of nerve and of muscle is known to fall off with time following the injury, although the fall is by no means as rapid as the subsidence of the ovulation potential. On the other hand, most observations of the injury potential have been made in excised tissues rather than in those provided with normal circulation.

What is the essential change in the stimulated ovary which leads to the generation of an ovulation potential? It is intimately dependent upon a normal blood-supply. Is it simply engorgement with blood and quickening of the circulation; is it associated with more active metabolism in the ovary at this time; or does it represent some more specific change? It will be important to follow more closely the time-course of the development of this internal electrical state. It will also be of great interest to determine whether atretic follicles differ electrically from normal follicles which would rupture eventually. In the course of the experiments described above, we have observed repeatedly that some follicles give greater potentials than others when the electrode is placed on their surface (cf. fig. 3) or when their rupture is induced by increasing the internal pressure in the ovary, etc. We suspect that these differences may be correlated with the condition of the follicle. It is possible that artificial rupture of an atretic follicle will not produce an ovulation potential or can, at best, produce only a very feeble one. This point must be tested by further experimentation.

In any case, the ovulation potential is empirically closely associated with the rupture of a ripe follicle; and in the case of the rabbit, at any rate, is large enough and sufficiently characteristic to be used as an external sign of ovulation. This in itself may make the potential a valuable tool for studies of the physiology of ovulation. Whether it can be similarly employed with the larger animals, in which the electrical phenomena originating in the ovary must necessarily be electrically shunted more effectively by the greater mass of body tissues, is another question which remains for future experiments to decide.

SUMMARY

Ovulation in the rabbit is associated with an abrupt change of electric potential between the vagina and the abdominal wall. This confirms the observation of Burr, Hill and Allen. The vagina becomes more positive by 5 to 12 millivolts and then slowly returns to its original potential in from five to twenty-five minutes. Direct observation shows the initial

deflection to be simultaneous with or a few seconds after rupture of the follicle (p. 726).

Artificially induced rupture causes similar electrical effects provided the ovaries remain *in situ*. This change of potential is not obtained either by aspirating the follicular fluid or by dropping it into the peritoneal cavity. A "peritoneal reaction" of 1 or 2 millivolts, opposite in sign and distinct from the ovulation potential, appears following these and other forms of contact with and possibly trauma to the peritoneum (fig. 2). Ovulation in an externalized ovary, electrically insulated except for its blood-supply, gives no ovulation potential.

The external surface of a ripe follicle is electrically more negative than the neighboring surface of the ovary (fig. 3). When a micro-pipette electrode is inserted into an ovulating ovary it registers at first from 2 to 15 millivolts more negative than when in contact with the surface of the ovary. This potential difference gradually subsides, like the ovulation potential (fig. 4). This effect is dependent upon an active blood-supply and is not found in kidney, abdominal muscles, intestine or a non-stimulated ovary.

REFERENCES

BURR, H. S., R. T. HILL AND E. ALLEN. Proc. Soc. Exper. Biol. and Med. **33**: 109, 1935.

BURR, H. S., C. T. LANE AND L. F. NIMS. Yale J. Biol. and Med. **9**: 65, 1936.

REBOUL, J., H. B. FRIEDGOOD AND H. DAVIS. This Journal **119**: 387, 1937.

ABSORPTION OF INSULIN FROM THIRY-VELLA LOOPS OF THE INTESTINE IN NORMAL AND DEPANCREATIZED DOGS

JOHN R. MURLIN, RUTH LATTA TOMBOULIAN AND H. B. PIERCE

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication June 26, 1937

A preliminary report under this title was made at the Ann Arbor meeting of the American Physiological Society in 1928. The completion of the work there recited was interrupted by the absence of the junior author from the laboratory, but was resumed on her return in 1932. The experiments reported in this paper were accomplished between that date and December, 1933. Altogether nearly 100 experiments on 4 dogs are here summarized.[1]

It is not necessary to review fully in this place the various attempts which have been made to induce the alimentary absorption of insulin. A fairly complete review has been published recently by Blotner (1936). This laboratory has been interested in the problem since 1923 (Murlin, 1923; Murlin, Sutter, Allen and Piper, 1924), while the last previous publication on the subject appeared in 1933 (Eaton and Murlin) and dealt with absorption induced by certain basic salts which buffer the acid reaction of the stomach and thereby permit insulin to survive peptic digestion. The present experiments were undertaken in the hope of analyzing more intimately the essential factors concerned in the absorption of a protein molecule so large as that of insulin. The fact of absorption can no longer be doubted. Recent notable proofs outside this laboratory have been furnished by Walton and Bassett (1935), and Lasch and Schönbrunner (1936). This study was actuated also especially by the negative findings of Bollman and Mann (1932). These authors found that direct enteral administration of insulin to normal dogs, some of which were quite similar in character to the experiments reported here, gave positive evidence of absorption only exceptionally. They prepared ileal loops of the intestine and when these were ready for experiment, after washing thoroughly with water, introduced 100 units of insulin in a volume of 5 cc. In only one case was there a "doubtfully significant" reduction in blood sugar. The

[1] Our attempts to induce the absorption of insulin from the alimentary tract have been aided for a number of years by a small annual grant from the Committee on Therapeutic Research of the American Medical Association to whom our best thanks are due.

authors do not state how many experiments were performed. They do admit, however, that "insulin in more concentrated solutions causes a definite lowering of the blood sugar but symptoms of hyperglycemia were not produced." The concentration at which a definite lowering of blood sugar could first be recognized, is not stated. Bollman and Mann found that insulin does disappear from an isolated ileal loop, and they estimate that 90 per cent had become "inactivated" by the mucosa within one hour. They recovered a sufficient amount of insulin (from 100 units introduced) to produce a marked fall in the blood sugar of a dog when injected subcutaneously. Incubation of insulin for 1½ hours with washings from such a loop in which it had been allowed to remain for five minutes, did not destroy the insulin. It appeared, therefore, that insulin is not digested by any protease produced in the ileum.

It seemed probable that the reason for Bollman and Mann's negative results in loop experiments might lie in the choice of the ileal region of the intestine rather than the jejunal, which was chosen in our preliminary series reported in 1928. It has been abundantly proved that the upper portions of the small intestine have greater powers of absorption than the lower. For this reason the upper jejunal portion just below the ligament of Treitz was chosen also for this series. The loops can be prepared without interference with pancreatic and bile ducts, or without undue stretching or traction on the hepatic ligament, and yet they lie closely aboral to the duodenum, and therefore present a more favorable site for absorption than does the ileum.

EXPERIMENTAL. The four dogs on which these experiments are based were operated for production of upper jejunal Thiry-Vella fistulae as follows: dog I, November 28, 1932; dog II, September, 1932; dog III, September 1932;[2] dog IV, late in December 1932. Dogs III and IV were depancreatized at the following dates: dog III, February 8, 1933; dog IV, October 16, 1933.

Extraordinary precautions have been taken in this series of experiments to avoid possible injury to the mucosa of the loops. Not in all cases were the ends of the loops everted, but when they were great care was taken to see that the loops were not employed for absorption experiments if any injury appeared on the visible portion of the mucosa. Also soft rubber catheters were employed for washing out the loops. As a rule the procedure for each experiment was as follows: The dog was placed on its back on an operating table. The loops were carefully washed out with warm water until no mucous strings or flecks could be obtained. The water was then drained out and the insulin solution or a preparatory

[2] These two dogs, II and III, were operated by Doctor Angevine in the Department of Biochemistry. We are indebted to that department for the gift of these animals.

wash introduced. If the latter was used it was allowed to remain for varying lengths of time, when it in turn was drained out and the insulin either as a simple aqueous solution or dissolved with various other substances in water, was introduced. Previous to washing, the skin about the mouths of the fistulae, while it still was dry, was thoroughly greased with petrolatum so as to avoid any possible absorption of insulin at the junction of the skin with the mucosa, in case of spillage. The insulin solution was held in the loops for the most part by means of the fingers of the observer pressed over the mouths. This proved to be a more effective and conservative means of closing the loop than any appliance we could devise by means of finger cots or glass cannulae, and a great many were tried. None of the appliances could be made perfectly watertight against a powerful peristalsis without employing so much pressure as to injure the mouth and thereby open the possibility of absorption of insulin through the injured tissue.

TABLE 1

Distribution of experiments

DOG NUMBER	TOTAL EXPERIMENTS		"SUCCESSFUL"		PERCENTAGE	
	Normal	Depancreatized	Normal	Depancreatized	Normal	Depancreatized
I	37		25		78	
II	8		6		75	
III	18	9	9	5	50	55
IV	19	5	10	5	58	100
	82	14	50	10	Av. 61	Av. 73

In the great majority of cases the insulin solution was not allowed to remain in the loop longer than about 20 minutes; in a few instances as long as one hour. In case of leakage of the insulin solution onto the surface of the animal's skin, it was immediately mopped up with absorbent cotton.

RESULTS. In table 1 is shown the distribution of experiments among the four dogs while they were normal and between the two which were later depancreatized. Out of 82 experiments on normal dogs, 50, or 61 per cent, were successful in the sense of causing a significant drop of blood sugar (12 mgm. per cent) within 30 minutes and remaining below normal for at least one hour. The maximal reduction on the average was nearly twice this amount and often much greater. As noted in previous publications from this laboratory on alimentary administration of insulin hypoglycemic shock was never observed.

Dog I which was used for the largest number of experiments scored

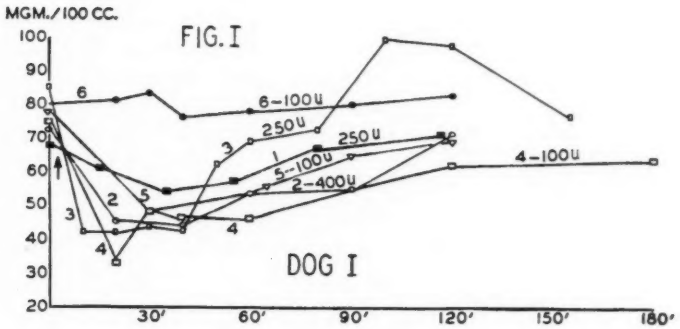
the largest percentage of successes. Dog III, as the figures stand, gave the smallest percentage, but it should be noted that the experiments on the different dogs were not all conducted in the same manner. Therefore, the figures are not correctly representative of individual variation. Only 14 loop experiments were performed on the two depancreatized dogs but the percentage of successes was greater than in normal dogs. This contrast probably is representative of a difference in susceptibility. Dog IV in five trials gave five successful effects on blood sugar and all were large effects.

Charts I and II present a few of the most successful effects for normal and depancreatized dogs, respectively. The criterion of a successful result on the depancreatized dog adopted here is a drop of at least 20 mgm. per cent within 1½ hours and a continued fall for 2 to 4 hours. The beginning of a return toward pre-insulin level is not directly related to the extent of the blood sugar fall.

Dosage. It is evident from the charts that the extent of the effect obtained by transmucosal absorption is not in these experiments definitely proportional to the amount of insulin introduced. The loops always were carefully washed with warm water so as to render them slightly hyperemic before the insulin was introduced. Variation therefore is not accounted for by secretion residues. The smallest dose which proved clearly effective was 35 clinical units. Twenty units, tried several times, were not often sufficient. Many successful effects were obtained with 100 units. With larger doses (chart I) there was at times a more prolonged hypoglycemia, but not always. Contrary to the experience of Bollman and Mann with ileal loops, 100 units or less in these experiments were effective quite as often as larger doses. The number of experiments at each dose level, however, was not equalized and this result may have been achieved only because, as experience was gained, it was found unnecessary to use larger doses. It is not intended to convey the idea that if the experiments had been equally distributed as to dosage and subjects no quantitative relation between quantity and effect would have been found.

Aids to absorption. When this work was started it had been observed previously in this laboratory that bile placed in a Thiry-Vella loop can aid absorption of insulin. Stimulated by the observation of Guyer and Claus (1928) that ox-bile placed in the stomach of guinea pigs previous to the administration of insulin may induce sufficient absorption of the latter to produce hypoglycemic shock, three students[3] performed the following experiment in November 1928. Three dogs with Thiry-Vella fistulae were available. Each dog was given successively on different days: 1,

[3] F. D. Nance, R. J. Thomas and L. Z. Zeidberg.



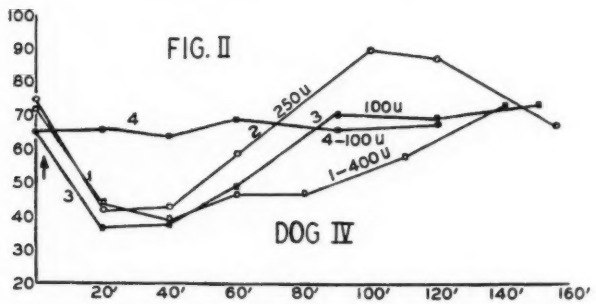
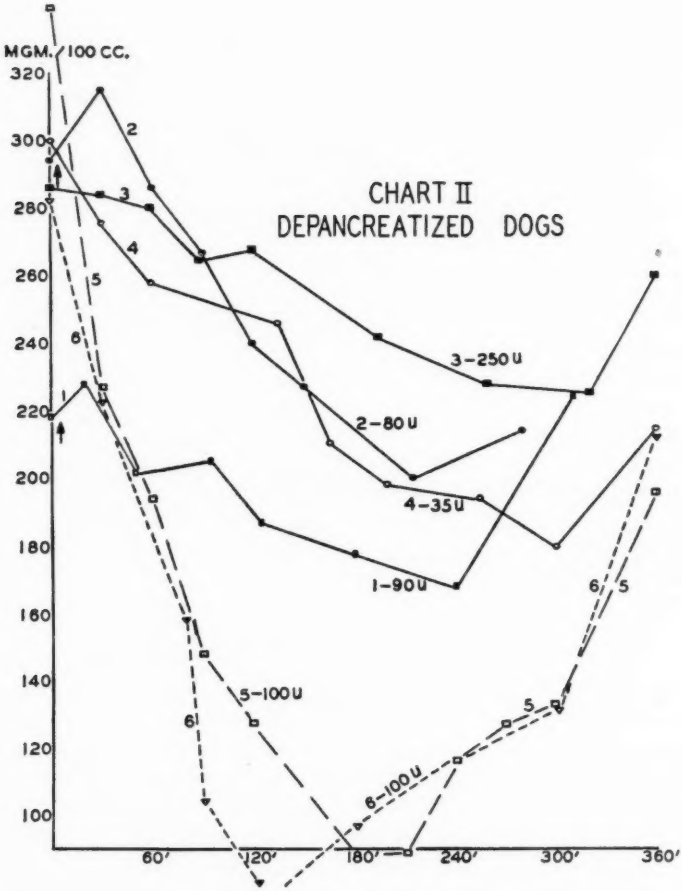


CHART I NORMAL

Chart I, fig. I. Successive curves of blood sugar obtained with dog I, curve *1*, 250 units of insulin in N/20 $NaHCO_3$ following wash with warm water; curve *2*, 400 units in N/20 $NaHCO_3$ and 1:1000 hexylresorcinol following preparatory wash with 10 per cent bile; curve *3*, 250 units in N/20 $NaHCO_3$ + 0.1 gram Na_2HPO_4 following preparatory wash with 10 per cent bile; curve *4*, 100 units in 5 cc. distilled water following preparatory wash with 0.1 per cent HCl (a very similar curve was obtained 10 mo. later following the same treatment and dosage); curve *5*, 100 units in 0.2 per cent HCl following preparatory wash with warm tap water; curve *6*, 100 units after incubation 20 hours at 37° with 5 cc. intestinal juice obtained from transplanted intestinal segment (Nasset, Pierce and Murlin, 1935) and following preparatory wash with 0.1 per cent HCl.

Chart I, fig. II. Successive curves of blood sugar obtained with dog IV.

Curve *1*, 411 units of insulin in N/20 $NaHCO_3$ + 0.1 gram $CaHPO_4$ following preparatory wash with 10 per cent bile; curve *2*, 250 units in N/20 $NaHCO_3$ + 0.1 gram $NaHPO_4$ following preparatory wash with bile; curve *3*, 100 units in distilled water following preparatory wash with 0.1 per cent HCl; curve *4*, 100 units after incubation for 20 hours at 37°C. with 5 cc. intestinal juice obtained from transplanted intestinal loop (see Nasset, Pierce and Murlin, This Journal 1935, **111**: 145) and following preparatory wash with 0.1 per cent HCl.



Curves *1–4* obtained with dog III; curves *5*, *6* obtained with dog IV. Curve *1*, 90 units insulin placed in loop in N/30 $NaHCO_3$ following thorough washing with warm tap water; curve *2*, 80 units placed in loop with 8 cc. blood serum + 0.5 gram $NaHCO_3$ and 0.1 gram Ca lactate following thorough washing with warm tap water; curve *3*, 250 units placed in loop with N/10 $NaHCO_3$ following preparatory wash with 10 per cent bile; curve *4*, 35 units placed in loop in N/10 $NaHCO_3$ (held 1 hr.) after preparatory wash with 0.5 per cent hexylresorcinol and 0.1 per cent HCl.

Curve *5*, dog IV, 100 units insulin placed in loop in distilled water (two weeks after pancreatectomy) after preparatory wash with 1:1000 hexylresorcinol and 0.1 per cent HCl; curve *6*, 1 week later 100 units in loop in distilled water following same preparatory wash (held 20 min.).

a certain dose of insulin in N/20 $NaHCO_3$; 2, the same following a dose of 10 per cent ox-bile which had been allowed to remain in the fistula for one-half hour; 3, the same dose of insulin dissolved in the 10 per cent ox-bile. In each case insulin *following* ox-bile produced a greater and more prolonged fall in blood sugar than insulin alone in the same strength of alkali or than insulin dissolved in the bile. The work was carefully executed.

It was evident from this experiment that a "preparatory wash" of a surface-lowering agent by effects on the mucosal cells might induce considerable absorption of insulin from the gut. This procedure of introducing a preparatory wash therefore became a standard one. There was

TABLE 2

Best combinations of wash and medium

WASH	MEDIUM	NUMBER EXPERIMENTS	
		Positive	Negative
Warm tap water	Distilled water	5	4
Warm tap water	N/20–40 $NaHCO_3$	4	3
Warm tap water	0.2 per cent HCl	2	0
10 per cent ox bile	$NaHCO_3$ + Ca salts	5	0
10 per cent ox bile	Hexylresorcinol + N/20 $NaHCO_3$	5	1
0.1 per cent HCl	Distilled water	5	0
0.1 per cent HCl	N/10–15 $NaHCO_3$	2	1
0.1 per cent HCl + hexylresorcinol	Distilled water	2	0
0.1 per cent HCl + hexylresorcinol	N/10–15 $NaHCO_3$	3	0
Total		33	9

of course no certainty that other agents than those which lower surface tension at interfaces might not also induce absorption.

One-tenth per cent HCl as a medium had been partially successful in some experiments with human subjects in 1924 (Murlin, Sutter, Allen and Piper). This solution was tried also as a preparatory wash, with some success. The best results, as judged by the extent and duration of the hypoglycemia, however, have been obtained with hexylresorcinol. This agent is supposed to owe its bactericidal properties in part at least to its strong surface-lowering effect (Leonard, 1924). One of us (H. B. P.) had confirmed its remarkable effect on the action of lipase reported by Glick and King (1932) and reasoned that it might exceed bile in its favoring action on the absorption of insulin, which proved to be true. It has

been used both as a preparatory wash and as a constituent of the medium in which insulin was contained when placed in the fistula for absorption.

Illustrations of the various combinations of preparatory wash and medium which have given most promising results are contained in table 2. There proved to be no advantage in following an alkaline wash with an alkaline medium or an acid wash with an acid medium, but bile followed by alkali or by hexylresorcinol and alkali in the medium was one of the best combinations. Hexylresorcinol is soluble in alkali, above pH 6.8, and more and more insoluble below this point. Nevertheless it has a

TABLE 3

Reduction of blood sugar from insulin placed in Thiry-Vella loops after different kinds of preparatory wash

WASH	NUMBER OF POSITIVE EXPERIMENTS	AVERAGE MAXIMUM FALL IN BLOOD SUGAR	AVERAGE TIME	AVERAGE DOSE CLINICAL UNITS	NUMBER NEGATIVE EXPERIMENTS	AVERAGE DOSE CLINICAL UNITS
Normal dogs						
		mgm. per cent	*min.*			
Warm water	20	22.6	35.5	218	9	132
10 per cent ox bile	12	23.1	33.0	184	5	108
0.1 per cent HCl	11	18.4	29.0	72	3	70
0.1 to 0.5 per cent hexylresorcinol in 0.1 per cent HCl	12	24.6	33.0	64	4	65
Depancreatized dogs						
Warm water	3	78.0	140.0	60	0	
10 per cent ox bile	2	66.0	250.0	175	0	
0.1 per cent HCl	1	100.0	240.0	50*	0	
0.1 to 0.5 per cent hexylresorcinol in 0.1 per cent HCl	6	139.0	185.0	62	1	20

*This dose was repeated 3 times at intervals of 1 hour; all others were single doses.

favoring effect on absorption when incorporated in a wash of 0.1 per cent HCl as shown in the table. Quite possibly small amounts of the suspended drug would be absorbed into the cells even from such a medium. Certainly its astringent quality can be felt plainly in the mouth when contained in this strength of acid. One-tenth per cent HCl alone as a wash followed by insulin in distilled water promoted absorption, but not so consistently as when the hexylresorcinol was contained in it.

In table 3 are given some quantitative comparisons. It is evident that the hexylresorcinol suspended in 0.1 per cent HCl proved to be the most effective of any of the four preparatory washes on which a sufficient

number of experiments were done to make the differences significant (normal dogs). In all of these experiments except three the medium for the insulin was either distilled water or weak alkali (N/10–40 $NaHCO_3$). The three exceptions were cases in which insulin was placed in 0.2 per cent HCl. Two in 80 unit doses followed water alone as preparatory wash and one in 100 units followed M/1000 hexylresorcinol. All were positive in their effects. The number of experiments on depancreatized dogs is not great enough to warrant any conclusion other than that hexylresorcinol made the best showing in the diabetic animal as it did in the normal. Three of the six experiments were performed on each of the two depancreatized dogs which reacted quite differently (see chart II).

Supplementing the discussion of dosage above, it may be noted that, as shown in table 3, ineffective doses were smaller on the average than effective doses (one exception).

As a medium for presentation of insulin to the mucosal surface more successful and more consistent results were obtained with weak alkali than with weak acid. When a calcium salt (lactate, chloride or acid phosphate) was added there was no advantage over N/20–40 $NaHCO_3$ alone. Some of these experiments were being done simultaneously with those of Eaton and Murlin (1933) in which the addition of calcium salts as additional buffers proved advantageous in surmounting gastric digestion of insulin. There was not the same hazard in these intestinal loops.

Addition of hexylresorcinol to the weak alkali in which insulin was contained appeared to favor absorption when the same drug had not been used as a preparatory wash, but the number of experiments is too small to be quite certain from the loop experiments. In the paper following evidence from a different procedure is more convincing.

Effect of intestinal secretion. Bollman and Mann (1932) inferred from their experiments that insulin is not digested by any protease obtainable from washings of their ileal loops, but that it is "inactivated" in some other way by the mucosa when put into a loop. They do not suggest what the destruction mechanism might be. Having fresh intestinal juice from transplanted loops of jejunum (Nasset, Pierce and Murlin, 1935) available at the time a number of digestion experiments were tried by admixtures of 5 cc. of the fresh juice with different quantities of insulin.

The insulin preparation varied in strength of solution from 50 to 500 units per cubic centimeter and the total volume of the mixture subjected to incubation from 6 to 30 cc. as shown (table 4). Toluene in sufficient quantity to prevent bacterial activity was added in all tests. The experiments were designed only to determine whether it is possible to demonstrate destruction of insulin by incubation with a secretion known to con-

tain an active protease (Pierce, Nasset and Murlin 1935). The two negative tests for insulin are shown in chart I, figs. I and II. In each case a positive test is shown from an experiment identical in all particulars except the absence of predigestion (curves 4 and 6 in fig. I; curves 3 and 4 in fig. II). It is evident that with relatively small amounts of insulin incubation for 20 hours with 5 cc. of fresh intestinal juice may cause inactivation or at least reduction in the potency of insulin to less than the minimal effective dose. It requires much longer time than Bollman and

TABLE 4

Inactivation of insulin with intestinal juice

EXPERIMENT NUMBER	AMOUNT OF INSULIN	VOLUME	INCUBATION PERIOD	TEST FOR INSULIN
	units	*cc.*	*hrs.*	
1	1000	20	22.5	Positive
2	1000	20	25.5	Positive
3	1250	30	22.0	Positive
4	1250	30	22.0	Positive
5	1250	30	23.0	Positive
6	1250	30	26.0	Positive
7	500	10	20.0	Positive
8	500	10	20.0	Positive
9	100	7	20.0	Negative
10	100	10	20.0	Negative
11	500	6	24.0	Positive

Mann employed and longer than the period of time the insulin was kept in the loops in these experiments to effect this result.

SUMMARY AND CONCLUSIONS

The absorption of insulin from Thiry-Vella loops of the jejunum, as judged by reduction in blood sugar, was studied in four dogs, while normal, and two of them also after pancreatectomy. Positive evidence of absorption was obtained in 61 per cent of the trials with normal dogs and in 73 per cent of the trials with the depancreatized.

It was found that several different substances put into the loop as a preparatory wash before the introduction of insulin favored its absorption. The most successful were 0.1 per cent HCl, bile and hexylresorcinol. It is probable that bile and hexylresorcinol act in virtue of their surface-lowering effects. The most favorable medium in which to dissolve the insulin for presentation to the mucosal surface proved in these experiments as in the preliminary ones (Murlin and Latta, 1928) to be weak sodium bicarbonate (N/20–40).

There was no very close relationship between the concentration of insulin and the effect. The least concentration to produce a marked reduction of blood sugar was 35 clinical units in 10 cc. Doses over 100 units in this volume as a rule gave somewhat larger effects than doses less than 100.

The concentration of hexylresorcinol necessary to produce a favorable effect was from 0.1 to 0.5 per cent.

Insulin can be inactivated by exposure of insulin to intestinal juice at body temperature for 20 to 30 hours. Whether or not this is a true digestive destruction has not been determined.

REFERENCES

BLOTNER, H. Am. J. Med. Sci. **192:** 263, 1936.
BOLLMAN, J. L. AND F. C. MANN. Am. J. Med. Sci. **183:** 23, 1932.
EATON, A. G. AND J. R. MURLIN. This Journal **104:** 636, 1933.
GLICK, D. AND C. G. KING. J. Biol. Chem. **97:** 675, 1932.
GUYER, M. F. AND P. E. CLAUS. Proc. Soc. Exper. Biol. and Med. **26:** 65, 1928.
LASCH, F. AND E. SCHÖNBRUNNER. Arch. Exper. Path. u. Pharmakol. **182:** 452, 1936.
LEONARD, V. J. A. M. A. **83:** 2005, 1924.
MURLIN, J. R. Endocrinology **7:** 519, 1923.
MURLIN, J. R., C. C. SUTTER, R. S. ALLEN AND H. A. PIPER. Ibid. **8:** 331, 1924.
MURLIN, J. R. AND R. LATTA. This Journal **85:** 397, 1928.
NASSET, E. S., H. B. PIERCE AND J. R. MURLIN. This Journal **111:** 145, 1935.
PIERCE, H. B., E. S. NASSET AND J. R. MURLIN. J. Biol. Chem. **108:** 239, 1935.
WALTON, R. P. AND E. F. BASSETT. Arch. Internat. Pharmacodyn. **48:** 322, 1934.

THE EFFECT OF HEXYLRESORCINOL UPON THE ABSORPTION OF INSULIN FROM THE GASTRO-INTESTINAL TRACT OF DOGS[1]

R. G. DAGGS, W. R. MURLIN AND J. R. MURLIN

WITH THE TECHNICAL ASSISTANCE OF RUTH L. TOMBOULIAN

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication June 26, 1937

Since insulin is of a protein nature and thus is destroyed or inactivated by the proteolytic enzymes, many attempts at oral administration have failed. According to many authors another obstructing factor in the intestinal absorption of insulin is the large size of the molecule.

Definite drops in the blood sugar of depancreatized dogs can be obtained, however, when insulin is given orally provided one protects it against the action of pepsin. In human diabetics the pancreas is present and continuing to produce trypsin. If insulin is protected against the action of pepsin by the use of an alkali, it may well be in an optimum medium for the action of trypsin. Acid changes in the administered mixture are not sufficient to protect insulin from both pepsin and trypsin. Many devices have been tried in an attempt to guard insulin against proteolytic action. Gaebler and Murlin (1925) reported moderate though positive absorption in phlorhizinized dogs when insulin was administered in enteric coated capsules combined with malic acid, sodium oleate or an amino acid liberating hydrochloric acid. Gais (1932) demonstrated that crude extracts of "*Ascaris lumbricoides*" yielded a potent antiprotease fraction that was capable of protecting insulin against peptic and tryptic digestion. The extract, when given orally to humans, proved toxic. The antiprotease of blood serum is capable of partially protecting insulin against proteolysis. The blood serum itself also facilitates a more rapid absorption (Murlin and Hawley, 1927 and Eaton and Murlin, 1933). Eaton and Murlin showed that large doses of insulin given by stomach tube along with calcium lactate and sodium bicarbonate to depancreatized dogs caused a lowering of blood sugar, decrease in urinary sugar and a rise in R.Q. Harned and Nash (1932) concluded from their work with antiproteases derived from intestinal worms and insulin that the protection of insulin against proteolysis in the gastro-intestinal tract is of little practical use, the

[1] This work was aided by a grant from the Committee on Therapeutic Research of the American Medical Association.

main obstacle to absorption being the protein character of the large insulin molecule. Campbell and Morgan (1933) also believe that the failure of insulin to act when given orally is due to lack of absorption since it is not rapidly destroyed in the intestines.

In an effort to facilitate absorption by lowering surface tension bile acids have been tried but proved unsuccessful in most cases. Saponin, according to Walton and Bassett (1935), increased absorption in Thiry fistulas of dogs.

Since favorable effects have been reported with the use of proteolytic enzyme protection and others with absorption facilitation, it was thought the combination of the two principles might be effective. Hexylresorcinol is a very effective surface tension lowering agent. The absorption of large molecules from the intestinal tract depends to a great extent upon a lowered surface tension (Verzar, 1933). The purpose of the present work was to investigate the effect of insulin, administered orally in conjunction with small amounts of hexylresorcinol and sodium carbonate, on the blood sugar of normal (and depancreatized) dogs.

Experimental procedure. The normal dogs were maintained upon a diet of kitchen scraps. In all cases food was withdrawn approximately 16 hours prior to an experiment. Following two basal blood samples taken at intervals of 15 minutes from the saphenous vein, the dogs were given by stomach tube sodium carbonate, hexylresorcinol and insulin. The blood sugar was followed for two to three hours, samples being taken at intervals of ten to fifteen minutes for the first hour, but at longer intervals thereafter. Blood sugar was determined by Benedict's (1931) method, using the Folin-Wu filtrate. Powdered insulin supplied by Squibb, or concentrated solution made by Lilly was made into solution as needed. Tenth normal hydrochloric acid was added to the water solution of insulin to make it acid to litmus. Crystalline hexylresorcinol as prepared by Sharpe and Dohme was made into solution as needed.

Results. The results are expressed in blood sugar curves. The curves shown are typical of the successful experiments. It must be stated that only about one-half of the experiments showed a significant blood sugar drop of over 20 mgm. per cent.[2] Why all attempts did not show uniform results is unexplainable, since all conditions were rigidly controlled. The most probable variable was the psychological condition of the dogs which may have varied the adrenalin production in some cases. This point will be discussed later.

Chart 1 shows the results for three experiments on dog "D" where only 100 units of insulin were given. The small arrow on all of the charts indicates the time of oral administration by stomach tube. The mixtures indicated on each of the charts were always washed down with water to

[2] Observe that this standard is higher than used for normal dog in the preceding paper.

insure against dragging any insulin back up onto the mucous membranes of the throat, mouth or nasal cavity when the stomach tube was withdrawn. It is well known that insulin can be absorbed from these mucous membranes. The dog was always watched carefully for at least half an hour and if there was any indication of regurgitation the experiment was discarded. Charts 2, 3 and 4 give typical results of successful attempts when

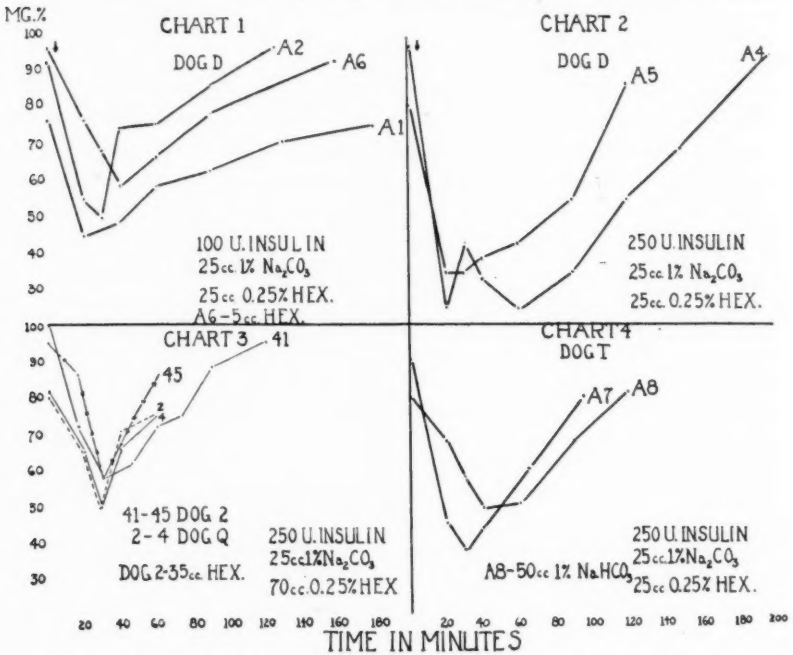


Chart 1. Three blood sugar curves obtained on the same dog on different days with 100 units.

Chart 2. Two blood sugar curves obtained on the same dog D as in chart 1, with 250 units of insulin.

Chart 3. Curves 2 and 4 obtained on dog 2 with 35 cc. 0.25 per cent hexylresorcinol; curves 41 and 45 obtained on dog Q with 70 cc. 0.25 per cent hexylresorcinol. Two hundred and fifty units insulin in all cases.

Chart 4 shows slightly less effect with 1 per cent $NaHCO_3$ than with 1 per cent Na_2CO_3.

250 units of insulin were administered in a single dose, with varying quantities of hexylresorcinol and alkaline salt.

Experiment A8 shown in chart 4 is included to illustrate that sodium bicarbonate can be used just as well as sodium carbonate. However, we have had more general success with the use of sodium carbonate.

Freshly prepared hexylresorcinol solutions did not seem to be as successful as ones that had stood for several months. The betterment due to aging may have been an oxidation process. If this were true, the addition of ferric chloride to the hexylresorcinol solution should simulate the aged solution. When sodium carbonate was added to a mixed solution of hexylresorcinol and ferric chloride a flocculent precipitate was obtained which was filtered out. The insulin was added to the solution just prior to administration. Chart 5 shows typical successful experiments using a

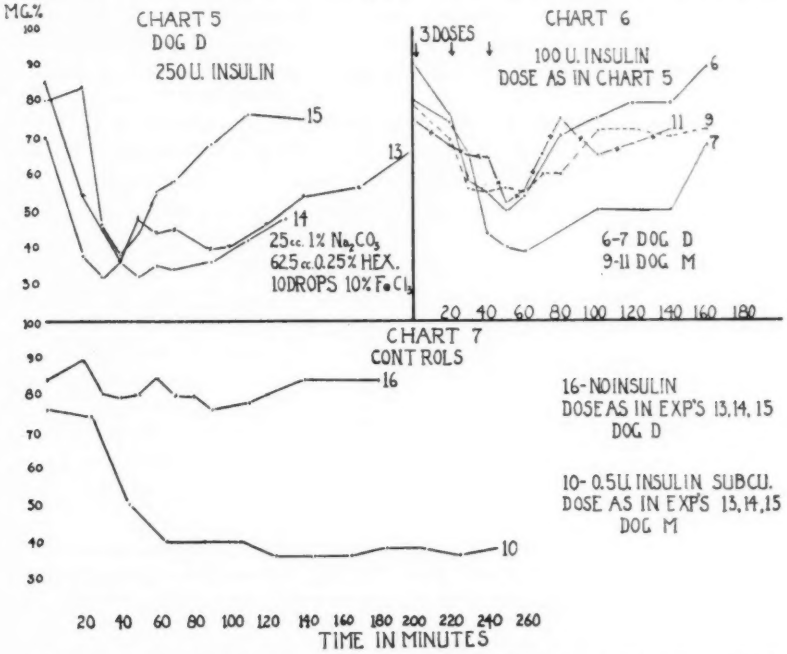


Chart 5. Typical blood sugar curves obtained with $FeCl_3$-treated hexylresorcinol.

Chart 6. Effect of 3 successive doses given at 20-minute intervals.

Chart 7. Curve 16 is a control without insulin; curve 10 the effect of the subcutaneous administration of 0.5 U. of insulin per kgm. of the mixture used orally in experiments 13, 14 and 15.

single 250 unit dosage of insulin in the presence of ferric-chloride-treated hexylresorcinol and sodium carbonate.

Even though the blood sugar often dropped to a level comparable with that when insulin is given subcutaneously, the blood sugar did not continue at this low level for so long as, in general, is the case with subcutaneous injections. A great part of the orally administered insulin is always lost or inactivated. Moreover, subcutaneously administered insulin is more

slowly fed into the general circulation, causing a more prolonged effect. The giving of three consecutive doses 20 minutes apart was tried in an attempt to maintain the low blood sugar values for a longer time. This was relatively unsuccessful as is illustrated in chart 6. There was no marked prolongation of the effect. Incidentally, this procedure seems to rule out the possibility of absorption from the mucous membranes of the throat or mouth.

Chart 7 gives examples of controls that could be repeated any number of times with practically identical results. Experiment 10 demonstrates the type of blood sugar curve obtained when this hexylresorcinol-sodium carbonate-insulin mixture was administered subcutaneously in a dose of

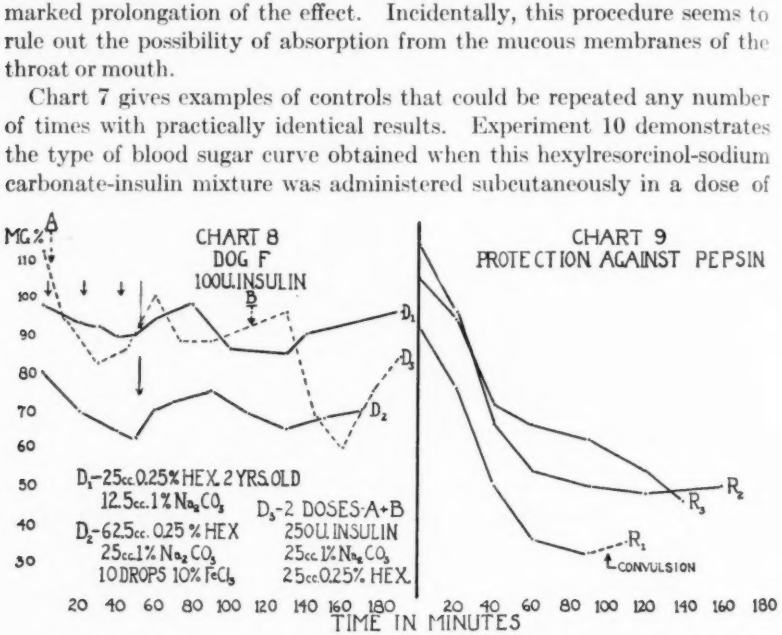


Chart 8. Repeated doses: D_1 of 2 year old hexylresorcinol; and D_2 of fresh hexylresorcinol "aged" by addition of $FeCl_3$. The long arrows mark points where the animal became excited; D_3, the result of two doses given at A and B, one hour and three-quarters apart.

Chart 9. Rabbit tests by subcutaneous injection of ½ unit of the mixture of hexylresorcinol, sodium carbonate and insulin subjected to pepsin digestion for ½ hour at 37°C. (R_2 and R_3); R_1, control with 0.5 unit per kilo of fresh insulin.

0.5 unit per kilogram, whereas experiment 16 proves that the blood sugar drops obtained with the oral treatment were not due to the hexylresorcinol-sodium carbonate mixture alone.

In order to illustrate the delicateness of this mechanism experiments D_1 and D_2 are cited in chart 8. The heavy long arrows indicate the time at which the animals were emotionally disturbed (fear and anxiety). The hump in the curve is probably due to the sudden production of epinephrin. D_3 shows a successful result with two doses (A and B) spaced farther apart.

A few experiments were conducted in order to throw some light upon the mode of action of hexylresorcinol. The ferric chloride-treated hexyl-

resorcinol-sodium carbonate-insulin mixture was incubated for ½ hour at 37°C. with pepsin (Merck powder) in the presence of hydrochloric acid with a resulting pH of 2.6. One-half unit per kilo of this incubated solution was then injected into rabbits and the blood sugar followed (chart 9). Curves R_2 and R_3 are duplicate experiments using the above procedure. Curve R_1 is the blood sugar curve obtained when ½ unit per kilo of insulin alone was given to a rabbit intraperitoneally. These experiments prove that this particuar mixture does protect insulin against the action of pepsin in vitro.

In vitro experiments were designed to test the effect of hexylresorcinol upon the tryptic digestion of casein. No appreciable effect was noted upon the rate of casein digestion as measured by formol titration. Hexylresorcinol therefore probably does not have a protective action against tryptic digestion of insulin. Hexylresorcinol more probably facilitates absorption either by lowering surface tension at the mucosal surface or by forming a combination product with insulin permitting a spacial rearrangement of the insulin molecule.

The intestines of one of the dogs that had received hexylresorcinol a great many times showed no gross or histological pathology at autopsy. The writers have been unable to find any evidence of serious toxic effects from the administration of hexylresorcinol by mouth either as a urinary antiseptic (Leonard, 1924) or as an antihelmintic (Lamson, Brown and Ward, 1932) when doses many times the quantity used in these experiments have been employed. Broom (1931) found the minimum lethal dose for the rat was 0.25 gm. per kilo.

SUMMARY

A solution mixture of hexylresorcinol, sodium carbonate and insulin given orally to normal dogs caused a significant, although not prolonged, drop in blood sugar in about one-half of the experiments.

REFERENCES

Broom, W. A. Brit. J. Exp. Path. **12:** 327, 1931.
Campbell, D. and T. N. Morgan. J. Pharm. and Exp. Ther. **49:** 450, 1933.
Eaton, A. G. and J. R. Murlin. This Journal **104:** 636, 1933.
Gaebler, O. H. and J. R. Murlin. J. Biol. Chem. **66:** 731, 1925.
Gais, E. S. Proc. Soc. Exper. Biol. Med. **30:** 216, 1932.
Harned, B. K. and T. P. Nash. J. Biol. Chem. **97:** 443, 1932.
Leonard, V. J. Am. Med. As. **83:** 2005, 1924.
Lamson, P. D., H. W. Brown and C. B. Ward. J. Am. Med. As. **99:** 292, 1932.
Murlin, J. R. and E. E. Hawley. This Journal **83:** 147, 1927.
Verzar, F. Nutr. Abst. and Rev. **2:** 441, 1933.
Walton, R. P. and E. F. Bassett. Arch. internat. pharmacodyn. **48:** 322, 1934.

REFLEX AND HUMORAL GASTRO-INTESTINAL INHIBITION IN UNANESTHETIZED DOGS DURING RECTAL STIMULATION[1]

WM. B. YOUMANS AND WALTER J. MEEK

From the Department of Physiology, University of Wisconsin, Madison

Received for publication June 26, 1937

Inhibition of the stomach and intestine of the dog by various types of stimuli in the colon and rectum has been demonstrated by several investigators. King (3) showed that mechanical stimulation of the anal canal, sudden high pressure in the rectum, and defecation produced a decrease in the tonus and motility of the intestine of the barbitalized dog. Pearcy and Van Liere (7) duplicated King's results and, in addition, found that distention of the colon resulted in decreased gastric tonus and inhibition of hunger contractions in the normal, conscious dog. Loew and Patterson (5) have shown that distention of the rectum inhibits the motility and decreases the tonus of the empty dog stomach. All of these workers have attributed the inhibition to reflexes dependent upon the splanchnic nerves. They have not considered the possibility of humoral inhibition except by mentioning the short latent period as an indication that adrenalin could not be the primary cause.

Recent studies indicate that physiologically active substances may be reflexly produced. Whitelaw and Snyder (8) have reported the production of sympathin during pseudo-emotional acitivity of decorticate cats. Liu and Rosenblueth (4) produced sympathin by stimulating afferent nerves. Partington (6) found that excitment, hypoglycemia, and cold, which are known to cause sympathetic activity, were accompanied by the production of sympathin. In each of these cases the substance produced was sympathin E or a mixture of sympathins E and I in which the former predominated.

In this study we have located the efferent pathways concerned with gastro-intestinal inhibition during rectal stimulation and have demonstrated that there is present during the inhibition a circulating, intestine-inhibiting substance which is not a product of the adrenal medulla.

METHODS. All records were taken from unmedicated dogs which were trained to lie quietly on the table without restraint. These animals, in

[1] Aided in part by grant from the Wisconsin Alumni Research Foundation.

most cases, showed no visible response to the rectal stimulation except by movements of the anal sphincter.

Records of stomach activity were obtained by having the animal swallow a balloon-tipped nasal catheter which was then connected with one arm of a water manometer, the other arm of the manometer being connected with a tambour. In some cases a mercury manometer was used as described below.

Jejunal activity was recorded by means of a balloon placed in a fistula. The balloon was connected by means of rubber tubing to one arm of a mercury manometer which floated a writing point on its other arm. The balloon and tubing were filled with enough water to record the tonus and the rhythmic activity of the fistula.

The term "rectal stimulation" refers to the insertion of a nasal catheter a few centimeters past the anal sphincter into the rectum and moving it to and fro sufficiently to elicit activity of the sphincter. It is not necessarily meant to imply that the sensory endings involved in the reflexes studied are located in the walls of the rectum itself.

Animals were prepared having normal Thiry or Thiry-Vella fistulae. Others were prepared having denervated fistulae, in which case the mesentery was cut and the blood vessels to the intestinal loop cleaned of nerve fibers and painted with phenol. Animals thus prepared withstood distention of the fistulae for seven days without loss of appetite or any apparent effects, whereas Herrin and Meek (2) have shown that similar distention of a fistula having its extrinsic nerves results in the symptoms characteristic of intestinal obstruction and death on about the eighth day. These animals also were not apparently conscious of pressures of 170 to 220 mm. of mercury in the denervated fistulae, while pressures of 70 to 100 mm. of mercury in normal fistulae caused the animals to show signs of discomfort.

Interruption of the parasympathetic pathways to the stomach and jejunum was accomplished by resecting a rib and sectioning the vagi above the diaphragm. Sympathetic pathways were interrupted by splanchnecotomy and removal of the lumbar sympathetic chains in successive operations through lumbar incisions. Animals were deprived of adrenal medullary tissue either by demedullation of both adrenals by cautery or by removal of one entire adrenal gland and demedullation of the remaining one. All operations were done under aseptic conditions and the animals were allowed time for complete recovery before records were taken.

Results. Gastric responses to rectal stimulation: The effect of rectal stimulation on the activity of the stomach was studied in 5 normal dogs. In 8 of 10 records decreased tonus and virtually complete inhibition of motility resulted. In the other 2 cases there was no fall in tonus, but there was partial inhibition of motility. Figure 1 illustrates the typical

response. The duration of stimulation was from 1 to 3 minutes, and the time from the beginning of stimulation to the onset of inhibition varied from a few seconds to 45 seconds.

Four records taken from two dogs, each of which had both vagi cut and the adrenal medullary tissue removed, showed inhibition of hunger contractions with no significant effect on gastric tonus. This is illustrated in figure 2.

In three dogs which were splanchnecotomized and had the lumbar sympathetic chains removed, rectal stimulation failed to inhibit the digesting or empty stomach. Eleven trials were made. See figure 3.

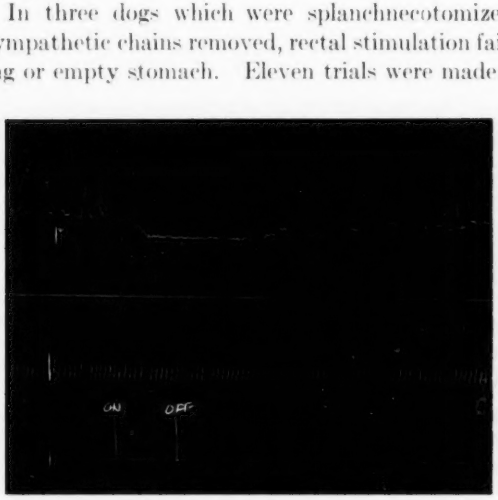


Fig. 1

Fig. 2

Fig. 1. Effect of rectal stimulation on the stomach activity of a normal dog. Reading from above downward the record shows stomach activity by balloon-mercury-manometer-tambour system, zero pressure line, time in 5 second and 1 minute intervals, and beginning and end of rectal stimulation.

Fig. 2. Effect of rectal stimulation on the activity of the stomach and of a denervated fistula of a vagotomized dog having one adrenal gland removed and the other demedullated. The upper record shows stomach activity. The period from the beginning of the first hunger contraction on the left to the arrow marks the length of the longest interval occurring during a 35 minute normal record preceding the rectal stimulation.

Jejunal responses to rectal stimulation. Inhibition of the motility of a jejunal fistula during rectal stimulation was recorded in each of 46 experiments on 9 dogs which were normal except for the fistulae. Inhibition was complete in 40 cases, and decreased tonus occurred in 41 cases. Four of the animals were sufficiently sensitive that merely introducing the catheter 3 or 4 cm. past the anal opening or withdrawing it resulted in a period of complete inhibition of the fistula. This is illustrated in figure 5. The two least sensitive animals showed only partial inhibition after a one minute period of rectal stimulation.

In 10 experiments the records were taken so that the period from the beginning of stimulation to the appearance of complete inhibition could be measured accurately. It was found to vary from 7 to 30 seconds. The latent period varies since the insertion of the catheter is arbitrarily taken as the beginning of stimulation, although it is more likely that the effective stimulus is associated with activity of some kind in the anal sphincter. This may follow insertion of the catheter at varying intervals. The shortest latent period may thus actually be less than 7 seconds.

Evidence that the effective stimulus is associated with the anal sphincter has been indicated as follows. If the stem of a glass funnel is inserted 3 cm. past the anal opening, sphincter activity may be observed accompanied by intestinal inhibition. The latter, however, gradually disappears, the motility returning to normal. The rectal mucosa may now

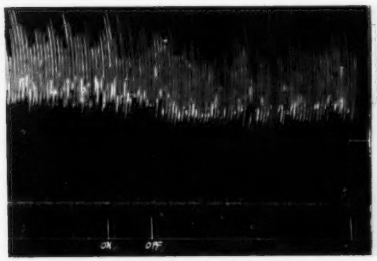


Fig. 3

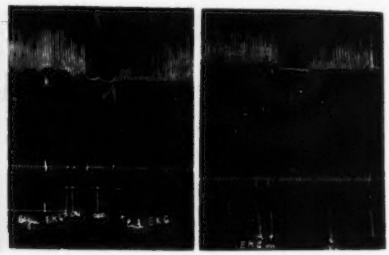


Fig. 4

Fig. 3. Failure of rectal stimulation to inhibit hunger contractions of a dog having its splanchnic nerves cut and lumber sympathetic chains removed.

Fig. 4. Effect of rectal stimulation (between *ON* and *OFF*) on the activity of a jejunal fistula of dog XI before vagotomy (left) and after vagotomy (right).

be stimulated with the catheter without affecting the sphincter and without causing intestinal inhibition.

Infiltration of the mucosa of the anal canal with novocaine does not alter the intestinal effects of rectal stimulation. This indicates that the effective stimulus involves endings in the muscle rather than in the mucosa.

The shortness of the latent period in some cases as well as the suddenness of the onset of inhibition (fig. 5) indicates that the inhibition is not primarily humoral. However, the duration of the inhibition for 1 to 6 minutes after the end of stimulation and the gradual return to normal activity suggests the gradual disappearance of an inhibitory substance.

Two of the animals used to demonstrate jejunal inhibition during rectal stimulation were vagotomized without in any way altering their responses to the stimulation. This is illustrated in figure 4. On the

other hand, no inhibition of the jejunal fistula resulted from rectal stimulation of a dog having its splanchnic nerves cut and lumbar sympathetic chains removed.

The above results show that inhibitory impulses are conveyed by means of sympathetic pathways to the walls of the small intestine, and possibly to the stomach, during rectal stimulation. There is no indication that the vagal fibers play any part in the gastro-intestinal inhibition thus produced.

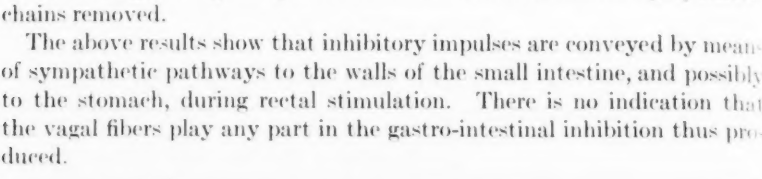


Fig. 5. Effect of rectal stimulation on the activity of a jejunal fistula having its extrinsic nerves intact. At the time indicated by the first arrow the catheter was inserted into the rectum. It was then left inserted without being moved except by sphincter activity and at the time marked by the second arrow the catheter was removed.

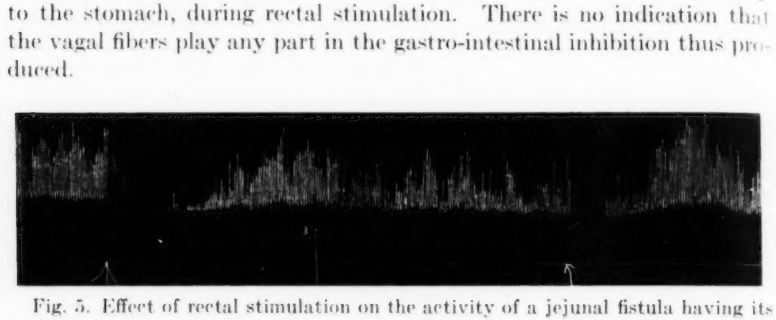


Fig. 6. Effect of rectal stimulation on the denervated jejunal fistula of dog VII. From left to right records show effect before adrenal demedullation and vagotomy, effect after removal of one adrenal gland and demedullation of the other, and effect after vagotomy. From left to right the records show the effects, first, before adrenal demedullation and vagotomy, second, after removal of one adrenal and demedullation of the other and third, after vagotomy.

Evidence of circulating sympathin I during rectal stimulation. Although the inhibition of an intestinal loop by rectal stimulation was prevented by sectioning the sympathetic pathways, yet inhibition of entirely denervated loops of the intestine was produced by rectal stimulation in 24 experiments on 4 dogs. The intestinal inhibition was complete in 3 of the 4 dogs. An example is illustrated in figure 6. These records differed as follows from those obtained from normally innervated fistulae:

1. The interval from the time of insertion of the catheter to the first evidence of inhibition of the denervated fistula varied from 20 to 80 seconds. 2. Sudden onset of complete inhibition did not occur in any case. 3. In many cases the maximal inhibition occurred in the 30 second period following the end of a 1 to 2 minute period of rectal stimulation.

Each of two animals having denervated fistulae were deprived of their adrenal medullary tissue by removal of one adrenal gland and demedullation of the other gland. These operations did not reduce the degree of intestinal inhibition resulting from rectal stimulation as determined in nine trials. An illustration is shown in figure 6. One of these animals was vagotomized, yet the same degree of inhibition of the denervated fistula resulted from rectal stimulation as occurred before adrenal demedullation and vagotomy. This is illustrated in figures 2 and 6.

Discussion. Cannon and Rosenblueth (1) have presented evidence that adrenergic fibers of the autonomic nervous system liberate a chemical mediator which, depending upon the effector organ in which it is released, may unite either with an "E" substance or an "I" substance. The resulting substances, which they call "sympathin E" in the former case or "sympathin I" in the latter, may diffuse from the place produced into the circulation and have exclusively excitatory or exclusively inhibitory effects, respectively. Sympathin I has been demonstrated to be in circulation after stimulation of various inhibitory sympathetic fibers (1). However, a difficulty in demonstrating the production of sympathin I by the stimulation of efferent nerves is that sympathin E is also produced due to the presence of vasoconstrictor fibers. It is to be expected that an inhibitory reflex depending upon sympathetic pathways for its efferent limb should be accompanied by the production of sympathin I in an almost pure form.

All of the data obtained in this study are in accord with the view that a substance is released as a result of rectal stimulation which corresponds to the description of sympathin I. Insertion of a catheter into the anal canal stimulates receptors from which impulses are carried to centers in the central nervous system. Efferent impulses pass to the intestine, and presumably to the stomach and colon, by way of inhibitory thoraco-lumbar autonomic fibers. At these endings a substance is produced which inhibits the smooth muscle locally and also diffuses into the blood. Within 20 to 80 seconds the inhibitory substance becomes evident in a distant denervated fistula as indicated by the onset of inhibition. The inhibitions we have studied in the intestine may thus be due either to the reflex production of an inhibiting substance at the site of the inhibition or to the circulatory removal of the substance to some other part of the intestine.

Doubtless the same statement is true of the inhibitions of gastric motility although the exact type of action was not demonstrated. The

intervals between gastric contractions were often so long that the latent period of the inhibition could not always be clearly shown to be less than a single circulation time. Humoral transmission of inhibiting substance could therefore not be excluded. No attempt was made to prove humoral transmission by an isolated denervated gastric pouch.

SUMMARY

Inhibition of the motility and decreased tonus of the stomach and of jejunal fistulae of the normal, conscious dog were recorded by the balloon method during mild mechanical stimulation of the anal canal and rectum.

Vagotomy or removal of the medullary tissue of the adrenal glands did not reduce the degree of gastro-intestinal inhibition resulting from rectal stimulation.

Inhibition of the stomach or jejunum could not be produced by rectal stimulation in animals having the splanchnic nerves cut and lumbar sympathetic chains removed.

Rectal stimulation produced, after a latent period of 20 to 80 seconds, complete inhibition of denervated jejunal fistulae in otherwise normal animals.

The degree of inhibition of denervated fistulae during rectal stimulation was not reduced by removal of the adrenal medullary tissue or by vagotomy.

These facts support the conclusion that an inhibitory substance is released at abdominal sympathetic endings, most likely in the walls of the gastro-intestinal tract, during rectal stimulation. This substance is produced in large enough quantities and is sufficiently stable to enter the blood stream and, after a complete circulation, inhibit a denervated jejunal fistula.

REFERENCES

(1) Cannon, W. B. and A. Rosenblueth. This Journal **104:** 557, 1933.
(2) Herrin, R. C. and W. J. Meek. Arch. Int. Med. **51:** 152, 1933.
(3) King, C. E. This Journal **70:** 183, 1924.
(4) Liu, A. C. and A. Rosenblueth. This Journal **113:** 555, 1935.
(5) Loew, E. R. and T. L. Patterson. This Journal **113:** 89, 1935.
(6) Partington, C. P. This Journal **117:** 55, 1936.
(7) Pearcy, J. F. and E. J. Van Liere. This Journal **78:** 64, 1926.
(8) Whitelaw, G. P. and J. C. Snyder. This Journal **110:** 247, 1934.

ACETYLCHOLINE CONTRACTURE OF DENERVATED MUSCLE[1]

G. C. KNOWLTON AND H. M. HINES

From the Department of Physiology, State University of Iowa

Received for publication June 26, 1937

The general subject of contracture has been reviewed by Gasser (1930). It is sufficient to recall here that the Vulpian-Heindenhain-Sherrington phenomenon and the acetylcholine contracture of denervated muscle have been identified as essentially the same response, the only difference being the source of the acetylcholine. The sensitivity of motor denervated muscle to "antidromic" stimulation at various times after denervation was studied by Heindenhain (1883). Similar studies on the response of such muscles to acetylcholine injections have apparently not been made.

The object of these experiments was to determine the quantitative aspects of the response of denervated muscle to acetylcholine in relation to the extent of atrophy, and to correlate these findings with the theories regarding the contractile mechanism of the contracture.

About 100 rats, ranging from 250 to 300 grams' body weight, were used in the experiments. The gastrocnemius of one side was denervated by sectioning the sciatic nerve, the opposite gastrocnemius serving as the control. At various times after nerve section studies were made concerning the sensitivity of the muscle to acetylcholine and the tension developed in response to 1, remote acetylcholine injection; 2, adjacent acetylcholine injection, and 3, direct electrical stimulation. Ether anesthesia was employed in all the experiments. Remote injections were made into the external jugular vein of atropinized, eserinized animals (0.1 mgm. atropine sulfate and 0.1 mgm. eserine sulfate intravenously). Adjacent injections were made into the femoral artery. In all instances the injection volume was either 0.1 or 0.2 cc. of a freshly prepared aqueous solution of acetylcholine bromide. Three to five minutes were allowed to elapse between injections. Direct electrical stimulation was applied through electrodes which pierced the muscle, one near the origin, the other at the tendon. Stimuli were delivered at the rate of 40 per second, either from the secondary of an inductorium or as condensor discharges. It was found that these two types of stimuli gave rise to tension responses of the same

[1] An abstract report of this work appeared in the Proceedings of the American Physiological Society, This Journal **119**: 353, 1937.

order of magnitude. Isotonic responses were recorded with a light weight lever giving a magnification of 20 to 1. Isometric responses were made against a Fick type torsion rod with optical recording which gave a magnification of 360 to 1.

The minimal amount of acetylcholine, given by remote injection, required to give a just detectable isotonic response was regarded as a measure of the sensitivity. Any muscle which failed to respond to an injection of 500 gamma of acetylcholine was regarded as "not sensitive." Acetylcholine, so given, in amounts up to one milligram never caused a contraction of the normally innervated muscle. The average minimal doses required at various times after denervation are given in column 1 of the table. It will be noted that the denervated muscle is not sensitive until between two and three days after nerve section when the sensitivity

TABLE 1

TIME AFTER NERVE SECTION	MUSCLE WEIGHT IN PER CENT OF NORMAL	MINIMUM DOSE A.C. IN GAMMA TO GIVE A RESPONSE (1)	TENSION IN GRAMS PER GRAM OF ORIGINA MUSCLE WEIGHT			
			A. C. injection			Electrical stimulation (5)
			Remote (2)	Adjacent (3)	Difference (4)	
days						
0	100	>500.0	0	459	459	1724
1		>500.0				
2		>500.0				
3	92	10.0				
7	79	0.2	137	433	296	1376
14	59	0.2	149	301	152	628
21	43	0.2	162	274	112	290
28	32	0.2	106	188	82	172
41	28	1.0	43	102	59	147

increases rapidly, reaching a maximum at about 7 days after denervation. This degree of sensitivity is then maintained for about three weeks after which a slow decline takes place.

The maximal tensions developed in response to adjacent and remote acetylcholine injections and electrical stimulations (columns 2, 3 and 5) are expressed in grams per gram of original weight, where the weight of the control muscle is taken as the original weight of the atrophic muscle. It may be seen that the tension response to remote acetylcholine injection remains practically constant from 7 to 21 days after denervation, that is, from the time that 25 per cent of the weight has been lost until over 60 per cent has been lost. During these periods of atrophy, however, the tensions resulting from adjacent injections and electrical stimulation show a progressive decrease. The observations of Brown (1937) indicate that

the response of denervated muscle to large amounts of acetylcholine administered by the adjacent route is a fusion of the quick contraction with the contracture response. The values for tensions (column 3) developed by denervated muscle to adjacent acetylcholine injections must then be a composite of the response of the quick contraction and contracture elements. The short volley of electrical stimuli employed in these experiments would presumably elicit a measurable tension of only the quick contraction elements of the muscle. The differences between tensions developed on adjacent and remote injections (column 4) observed in the different periods of atrophy parallel the changes in tension elicited by electrical stimuli. It should be pointed out that the electrical stimuli ordinarily cause a greater tension development in the quick contraction elements than that resulting from adjacent acetylcholine injections because of the difficulties encountered in the effective application of the latter type of stimulus.

It appears as if, by denervation, some element in muscle, which becomes very sensitive to acetylcholine, is made capable of yielding a constant contracture tension during a time in which the muscle loses approximately one-half of its weight. At the same time there is a progressive decrease of the quick contraction tension. These findings suggest that the contracture response elicited by remote acetylcholine injection involves a different contractile element of the denervated muscle than that responsible for the quick contraction induced by adjacent acetylcholine injection or by short volleys of electrical stimuli.

The nature of the contractile element sensitized to acetylcholine is a matter of speculation. Attempts have been made to identify it with the intrafusal fibers of the muscle spindle (van Dijk, 1935). An objection to such arguments has been the work of Hinsey and Gasser (1928) who found that the tension developed following acetylcholine injection and electrical stimulation were almost the same. It would be unbelievable that the intrafusal fibers present could exert such a tension. However, an examination of that work in the light of the more recent experiments of Brown, Dale and Feldberg (1936) shows that the acetylcholine injections were made in such a fashion that the responses probably represent both contraction and contracture elements. To stimulate the sensitized fibers exclusively resort must be had to remote acetylcholine injection.

If the contracture response is to be ascribed to the intrafusal fibers, then these fibers must undergo atrophy at a slower rate than do the extrafusal fibers. Sherrington (1894) thought that the intrafusal fibers did not atrophy following denervation while Tower (1932) has described their atrophy as coinciding with that of the bulk of the muscle. These results would indicate an atrophy rate lying between the two extremes cited in

that noticeable atrophy would seem indicated at the time of diminishing acetylcholine sensitivity and tension development.

It is possible, however, that the sensitized fibers are derived from the extrafusal fibers. Individual fibers of a muscle do not atrophy at the same rate. Some may show extensive atrophy while others are only beginning to be affected. If, at a certain stage of atrophy, the extrafusal fiber develops this peculiar sensitivity to acetylcholine it is quite possible that the total effective number of sensitive fibers would remain constant over a considerable period of time. This would only require that the rate of appearance of sensitized fibers should equal their rate of disappearance over the period of 7 to 28 days after nerve section.

SUMMARY

A study of the responses of normal and denervated rats' gastrocnemii to 1, remote acetylcholine injection; 2, adjacent acetylcholine injection, and 3, electrical stimulation has shown that:

The sensitivity to remote injection appears between two and three days after denervation. By seven days after nerve section a maximum is reached which is maintained up to 28 days when a slow decline of sensitivity sets in.

The response to remote acetylcholine injection, in terms of the grams tension per gram of original weight, remains practically constant for from 7 to 21 days after nerve section while that due to electrical stimulation and adjacent acetylcholine injection decreases.

From this it is concluded that the tension developed on remote acetylcholine injection is the result of activation of fibers physiologically different from the bulk of those activated by direct electrical stimulation. Some possibilities as to the precursor of these sensitized fibers are discussed.

REFERENCES

BROWN, G. L., H. H. DALE AND W. FELDBERG. J. Physiol. **87**: 394, 1936.
BROWN, G. L. J. Physiol. **89**: 438, 1937.
GASSER, H. S. Physiol. Reviews **10**: 35, 1930.
HEIDENHAIN, R. Arch. f. Physiol. (Suppl.) 133, 1883.
HINSEY, J. E. AND H. S. GASSER. This Journal **87**: 368, 1928.
SHERRINGTON, C. S. J. Physiol. **17**: 211, 1894.
TOWER, S. S. Brain **55**: 77, 1935.
VAN DIJK, J. A. Arch. neerl. de physiol. **20**: 317, 1935.

A RELATIONSHIP BETWEEN THE BLOOD SUGAR LEVEL AND THE RATE OF SUGAR UTILIZATION, AFFECTING THE THEORIES OF DIABETES

SAMUEL SOSKIN AND R. LEVINE

From the Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital[1] *and the Department of Physiology, University of Chicago*

Received for publication June 28, 1937

Without entering into a discussion of the relative merits of either theory of diabetes it will be generally conceded that, although each theory is supported by a large body of experimental evidence, each is also faced by certain well-established experimental facts which it cannot explain. The Non-Utilization Theory explains diabetes as a condition in which carbohydrate cannot be oxidized, largely upon the basis of its interpretations of the D:N ratio and the respiratory quotient. But this theory cannot explain the more direct observations of the utilization of large amounts of sugar by the diabetic organism, in such experiments as the following:

a. After hepatectomy of the depancreatized dog (Mann and Magath, 1923).
b. After hypophysectomy of the depancreatized dog (Houssay and Biasotti, 1931; Soskin et al., 1935b).
c. After evisceration of dogs which have been fasted to the point of "hunger diabetes" (Soskin and Mirsky, 1935a).

The Overproduction Theory is based upon different interpretations of the D:N ratio and the respiratory quotient (cf. Soskin, 1930). It is also supported by a more recent and independent line of evidence concerning the dextrose tolerance curve (Soskin et al., 1934b, 1934a, 1936). Since the Overproduction Theory postulates the uncontrolled formation of sugar by the liver, in excess of the amount which can be and is disposed of by the muscles, this theory does explain the appearance of evident sugar consumption in the diabetic organism, as soon as the liver is removed or whenever it is hindered in its capacity to form sugar. But the Overproduction Theory cannot explain the subnormal use of sugar by isolated tissues or organs of the diabetic animal, as observed in such experiments as the following:

[1] Aided by the Max Pam Fund for Metabolic Research.

a. Observations on the oxygen consumption and respiratory quotient of excised slices of muscle and kidney, from normal and depancreatized dogs (Richardson, et al., 1930).
b. The glucose and lactic acid balance during the perfusion of isolated hearts from normal and depancreatized dogs (C. Lovatt Evans et al., 1935b).

From the above and other evidence, it has been apparent for some time that neither the Non-Utilization Theory nor the Overproduction Theory could be wholly correct or entirely wrong. It seemed likely that these two theories were but two incomplete and complementary aspects of a more comprehensive understanding, which would resolve their apparent contradictions and preserve the usefulness of each. In seeking such a point of view our attention was drawn to a phenomenon which has been noticed by several investigators but not adequately evaluated by any, namely, the relation of the blood sugar level to the utilization of sugar. This relationship seems to be a fundamental one, since it appears in the results obtained, on various experimental organisms and under different physiological conditions, by Burn and Dale (1924), Cruickshank and Shrivastava (1930), Wierzuchowski (1931), Rubenstein (1932), C. Lovatt Evans et al. (1935a) and Soskin and Mirsky (1935a). It occurred to us that this relationship must be very important in any determination of the comparative rates of sugar utilization by phlorhizinized, normal and depancreatized animals, which usually maintain such widely differing blood sugar levels. We proceeded, accordingly, to compare the rate of sugar utilization of dogs in various states, over a large range of blood sugar levels. The present communication deals with our results on normal and depancreatized dogs respectively.

Methods. The utilization of sugar in our experiments was calculated from blood sugar, blood lactic acid and muscle glycogen data obtained after removal of the liver and intestinal tract and ligation of the ureters. All the dogs used in these experiments were fasted for three days and were eviscerated on the morning of the fourth day by a modification of the method for hepatectomy described by Markowitz, Yater and Burrows (1933). All operations and the subsequent experimental procedures were performed under nembutal anesthesia. The preliminary fast obviated the possible influence of variations in previous diet, and also served to facilitate the operative procedure and the post-operative survival. The three-day fasting period was necessary for the diabetic dogs and hence was used for all the animals in order to keep the time relationship constant. The diabetic dogs were depancreatized on the first day of fasting and eviscerated on the morning of the fourth day, no insulin having been given at any time.

Following the evisceration each animal received a continuous intra-

venous injection of dextrose, from a constant injection pump, at a rate varying from 1/32 to 1 gram/kgm./hour, depending upon the particular range of blood sugar level at which we wished to maintain it. After allowing two hours for recovery from the immediate shock of the operation and for the attainment of the desired blood sugar level, samples of blood for sugar (Somogyi, 1930) and lactic acid determinations (Friedemann and Graeser, 1933) and samples of two different muscles for glycogen assay (Good et al., 1933) were taken as nearly simultaneously as possible. Blood sugar sampling was continued at half-hour intervals and four hours later a final set of blood sugar, blood lactic acid and muscle glycogen samples was taken. Some experiments were terminated after a shorter period, if a decline in the physiologic state of the animal seemed impending. In no instance, however, have we used the data from any experiment in which the observation of sugar utilization extended over a period of less than two hours.

The details of our calculation are illustrated by the following typical example:

Normal dog, experiment 8. Weight 9.0 kgm. Experimental period 4 hours. Average blood sugar throughout the experiment was 80 mgm. per cent. Muscle mass calculated as 50 per cent of body weight (Best et al., 1926), equals 4500 grams. Blood and extracellular fluid calculated as $\frac{1}{6}$ of body weight (Burn and Dale, 1924; Lavietes, 1936), equals 1500 cc.

Initial average glycogen	0.42	per cent
Final average glycogen	0.28	per cent
Difference	0.14	per cent

Glycogen decrease $\frac{(140 \times 4{,}500)}{100}$ equals 6,300 mgm.

Initial blood sugar	96	mgm. per cent
Final blood sugar	80	mgm. per cent
Difference	16	mgm. per cent

Blood sugar decrease $\frac{(16 \times 1500)}{100}$ equals 240 mgm.

Initial lactic acid	39.2	mgm. per cent
Final lactic acid	88.2	mgm. per cent
Difference	49.0	mgm. per cent

Lactic acid increase $\frac{(49 \times 1{,}500)}{100}$ equals 735 mgm.

Dextrose injected during experiment equals	2,250 mgm.
Total sugar disposed of	8,790 mgm.
Minus the lactic acid increase	735 mgm.
Sugar utilized	8,055 mgm.

Sugar utilized per kgm. of original body weight per hour $\frac{(8,055)}{9 \times 4}$ equals ..... 224 mgm.

RESULTS. The essential data from which our results are calculated are given in table 1. The results are graphically presented and compared in figures 1 to 3.

Figure 1 presents the results in our normal dogs. The height of the cross-hatched blocks indicates rate of sugar utilization while the width of each block is proportional to the length of the observation, experiment number 1 being of 4 hours' duration. The continuous line above each block is a curve of all the blood sugar values obtained during that experiment. It may be seen that, with the exception of experiments 6 and 12, a line drawn through the tops of the blocks would give a remarkably smooth S-shaped curve. There can be no doubt that, between the limits of the initial and final plateaux, the rate of sugar utilization depends upon the height of the blood sugar level. When one considers the possibilities of individual variation inherent in our assumptions of muscle mass, body fluid, etc., the uniformity of our results is surprising and lends weight to their significance.

The extent to which our results may or may not be influenced by our experimental conditions is indicated by a comparison with some figures recalculated from the data of Wierzuchowski (1931) *based on oxygen consumption and the respiratory quotient in normal, intact, nembutalized dogs:*

AVERAGE BLOOD SUGAR LEVEL	WIERZUCHOWSKI (SUGAR OXIDATION)	OUR RESULTS (SUGAR UTILIZATION)
mgm. per cent	*mgm./kgm./hour*	*mgm./kgm./hour*
50	215	228
181	325	306
285	413	410

This striking parallelism shows that evisceration does not significantly alter the consumption of sugar during the time limits which we employed. It also indicates that our figures for sugar "utilization" in these experiments may be regarded as equivalent to sugar "oxidation" in the usually accepted sense.

Figure 2 presents the results in our depancreatized animals. The conventions are the same as for figure 1. These results show some quantitative difference from those obtained in the normal animals. But the utilization or oxidation of sugar persists, and the normal relationship

TABLE 1

DOG	NUMBER	WEIGHT	DURATION OF EXPERIMENT		TOTAL SUGAR INJECTED	BLOOD SUGAR*			BLOOD LACTIC ACID		MUSCLE GLYCOGEN	
						Initial	Final	Average	Initial	Final	Initial	Final
		kgm.	*hours*	*minutes*	*grams*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
Normal	1	10.20	3	42	1.001	35	8	17	52.50	125.1	0.435	0.245
	2	11.50	4	0	1.292	75	25	43	36.90	105.3	0.510	0.370
	3	9.00	4	0	2.250	96	80	80	39.20	88.2	0.420	0.280
	4	16.30	4	0	6.760	54	88	78	32.40	55.8	0.690	0.560
	5	7.50	4	0	5.040	75	167	128	22.70	77.6	0.380	0.230
	6	8.10	4	0	4.050	149	158	149	109.40	109.4	0.320	0.090
	7	11.00	4	0	11.908	182	309	238	54.50	128.3	0.365	0.255
	8	10.50	3	30	7.915	277	390	342	27.70	132.1	0.780	0.560
	9	15.40	2	15	10.918	271	369	342	95.40	130.0	0.530	0.425
	10	8.00	3	30	9.291	271	434	350	64.30	126.4	0.390	0.195
	11	7.75	4	0	10.964	416	365	393	39.10	103.9	0.475	0.330
	12	13.80	4	0	16.236	376	527	442	49.10	81.7	0.585	0.560
	13	8.60	3	30	13.558	315	568	445	50.90	128.2	0.510	0.290
	14	8.60	3	30	13.781	400	724	591	40.90	85.0	0.550	0.370
	15	15.60	3	13	19.665	610	738	616	42.20	124.2	0.725	0.560
Depancreatized	1	12.80	4	0	0.000	201	143	168	86.40	110.7	0.485	0.430
	2	17.20	3	0	4.107	206	201	187	109.30	170.1	0.270	0.200
	3	13.20	2	10	1.385	262	207	230	155.70	185.4	0.105	0.070
	4	9.70	3	25	4.893	239	282	262	115.00	198.0	0.270	0.130
	5	7.50	2	0	3.640	331	394	365	162.50	190.4	0.020	0.010
	6	12.20	2	0	5.319	318	460	385	72.50	163.8	0.285	0.210
	7	11.30	3	50	15.361	327	625	524	103.30	172.8	0.380	0.205
	8	10.50	3	9	13.280	493	594	601	118.80	199.8	0.400	0.265
	9	9.50	3	30	12.919	494	727	642	132.30	253.8	0.420	0.260
	10	15.60	3	15	23.902	543	878	709	168.70	210.1	0.430	0.180
	11	10.00	2	30	11.725	820	1008	926	113.40	182.5	0.390	0.240
	12	11.45	3	30	17.638	839	1058	952	79.10	161.1	0.585	0.400

* The figures for average blood sugar level are averages of all the blood sugar determinations, made at half hourly intervals, throughout each experiment.

between these processes and the height of the blood sugar level is maintained. Thus, though there is a quantitative difference, it is obvious that the fundamental processes of sugar use are not disturbed in the diabetic organism.

Figure 3 compares the results in the normal and depancreatized dogs.

The heavy continuous lines are smooth curves drawn through the scatter of points plotted for each group of animals, using the average blood sugar level for each experiment as abscissae. The lighter broken line marks the rate of sugar utilization in normal animals at the normal blood sugar level. The point at which this line crosses the curve for the depancreatized animals indicates the blood sugar level (somewhat over 300 mgm. per cent) at which diabetic animals use this same normal amount of sugar. It may be seen that the general relationship between blood sugar level and sugar utilization is almost identical whether the pancreas is present or not. This relationship, therefore, cannot be ascribed to any action of

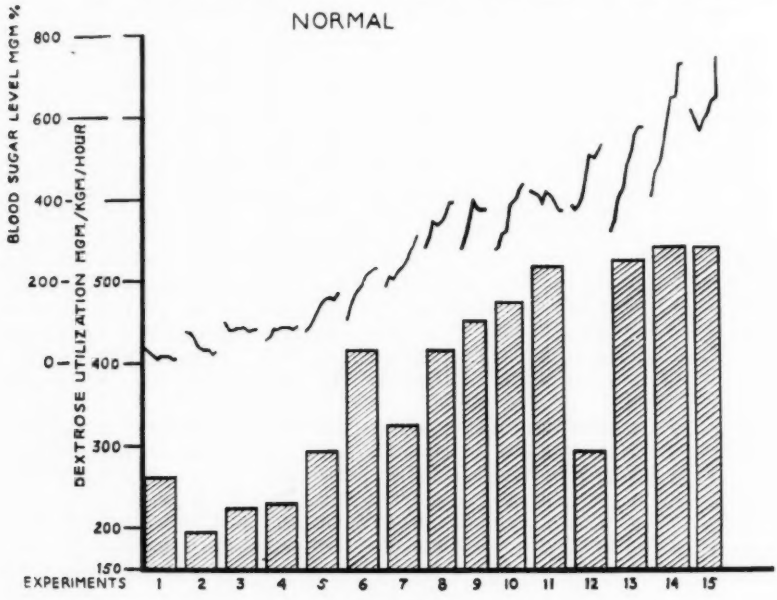


Fig. 1

insulin. Furthermore, a comparison of the two curves shows that the utilization of sugar by depancreatized animals is uniformly depressed throughout most of the blood sugar range, but rises to meet the normal rate at high blood sugar concentrations, even though insulin is entirely absent.

Discussion. We have shown that dextrose utilization in the depancreatized dog is qualitatively similar to that in the normal dog. In both types of animal, the rate of utilization depends upon the height of the blood sugar level. Within a wide range of blood sugar values, the diabetic dog utilizes less sugar than the normal dog at any particular glycemic

level. But above certain high levels this difference disappears, and both types of animal use the same amounts of carbohydrate at the same blood sugar levels. *When, however, one compares the rate of utilization of the normal animal at its usual normal blood sugar level with the rate of utilization of the diabetic animal at the hyperglycemic levels which it ordinarily maintains, it is apparent that the diabetic animal habitually uses as much or more sugar than the normal animal.*

In view of these results, insulin cannot be regarded as essential to the utilization of dextrose. It probably plays the part of a catalyst or acti-

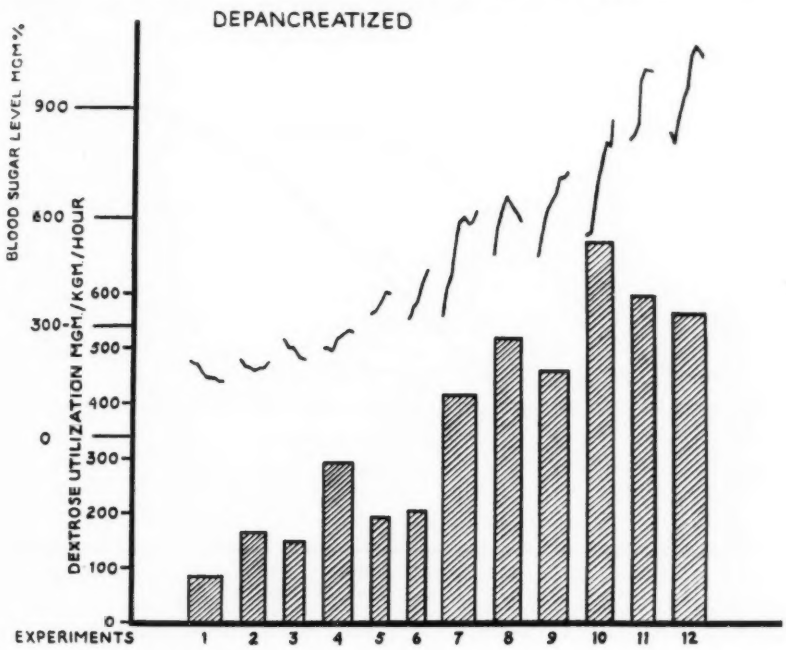


Fig. 2

vator in a process which can proceed at a slower rate in its absence. The similarity in shape of our utilization curves for both normal and depancreatized dogs, and the fact that these curves meet at the higher glycemic levels, suggest that insulin activates some phase of the blood sugar level → sugar oxidation sequence. It seems advisable to defer any further discussion of the manner and locus of insulin action to a more appropriate, forthcoming communication.

Our work confirms and explains both types of experimental evidence, which were pointed out in our introductory remarks as being inconsistent

with the Non-utilization and Overproduction Theories of diabetes respectively. The present results support the previous direct observations of the utilization of carbohydrate by the diabetic organism. But to the extent that the diabetic animal does use less sugar than the normal at the same blood sugar level, the Non-utilization Theory is not entirely without validity. From this point of view it is also easy to explain the previous observations of a diminished utilization of sugar by isolated diabetic tissues or organs. Thus Richardson et al. (1930) concluded that diabetic tissue oxidizes little or no sugar from a comparison of the use of sugar by normal and diabetic tissue slices at the same sugar concentration of 200

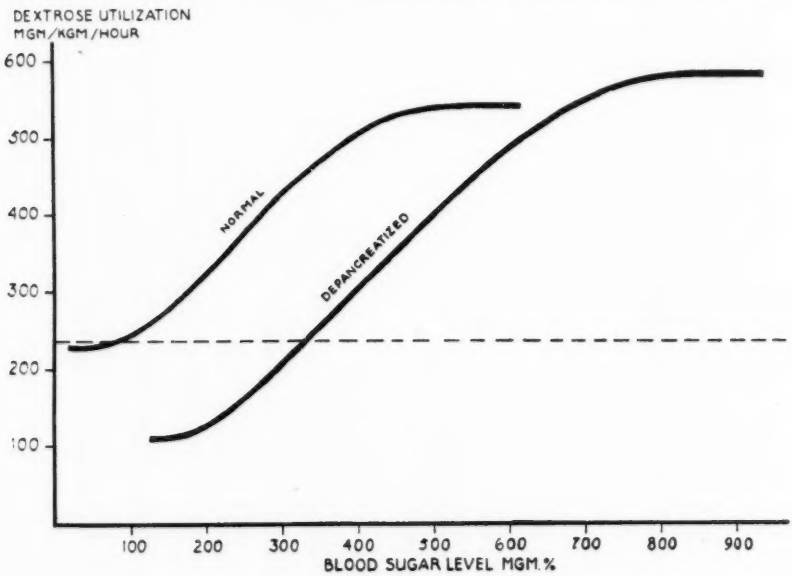


Fig. 3

mgm. per cent. Similarly, Lovatt Evans et al. (1935b) compared the sugar utilization of normal and diabetic hearts at the same diabetic blood sugar levels. It is clear from our work that these results depended upon the arbitrary selection of the experimental conditions under which the comparisons were made. For example, a reëxamination of the data of Lovatt Evans et al. in the light of our work, reveals the fact that their diabetic hearts at the diabetic sugar level used amounts of sugar of about the same order of magnitude as did their normal hearts at approximately normal sugar levels. This type of evidence, therefore, cannot be taken as proof for the Non-utilization Theory or as contrary to the Overproduction Theory of diabetes.

It is obvious that both "non-utilization" and "overproduction" play a rôle in diabetes. Which of these disturbances is to be regarded as the more important element, depends upon the point of view. Although the diabetic animal uses less sugar than the normal at the same blood sugar level, the latter condition does not ordinarily obtain. At its characteristically high blood sugar levels, the diabetic organism uses as much or more sugar than the normal organism at the usual normal blood sugar levels. Thus, diabetes is not a condition in which less sugar is utilized. From this point of view, the "non-utilization" component of diabetes would appear to be of minor significance. If, however, one wished to reason teleologically, the hyperglycemia of diabetes might be regarded as a compensatory phenomenon which enables the diabetic to utilize the normal amount of carbohydrate. From this standpoint the disability in utilization would assume the rôle of a primary cause, and the "non-utilization" factor (perhaps better called the "relative under-utilization" factor) in diabetes might be regarded as an important one. When, however, one puts aside teleological speculation and considers experimental and clinical facts, it is clear that "overproduction" is the major factor in diabetes. At its usual high blood sugar levels the diabetic animal excretes large quantities of sugar into the urine, develops a ketosis, gives the characteristic D:N ratio and respiratory quotient, and exhibits the clinical syndrome of polyuria, polydipsia, polyphagia, loss of weight, etc. But we have seen that *at these same high blood sugar levels* at which it is excreting large amounts of sugar, the diabetic animal is also using at least as much sugar as the normal animal under normal conditions. Hence, the glycosuria and the other characteristic experimental and clinical phenomena associated with diabetes cannot be ascribed to a lack of sugar utilization by the muscles, but must be due to the overproduction of sugar by the liver.

The present results show the necessity for re-examining much of the previous work on diabetes, and for repeating some of it. It is to be hoped that, by reconciling the two theories of diabetes, we shall have helped to end a long and spirited controversy which, while it has led to much useful work, has also resulted in a great waste of motion and emotion.

CONCLUSIONS

1. The completely depancreatized dog, in the absence of insulin, can utilize dextrose at any rate of which the normal animal is capable.

2. In both the normal and the depancreatized dog the rate of dextrose utilization depends upon the height of the blood sugar level.

3. Within a certain glycemic range, the depancreatized dog utilizes less sugar at any given blood sugar level than the normal animal at the same level.

4. *But, the depancreatized dog at its usual hyperglycemic level utilizes as much or more sugar than the normal dog at its usual normal blood sugar level.*

5. On the above basis, the two supposedly opposing theories of diabetes may be reconciled. The Non-Utilization Theory applies only in a particular restricted sense. The Overproduction Theory remains as the major component of the new conception.

6. Previous work on the comparative utilization of carbohydrate by the normal and diabetic organism must be reconsidered with due regard to the above facts.

Acknowledgment of valuable technical assistance is due to Miss LaDonna McEllhiney and to Miss Bernice Huddlestun.

REFERENCES

BEST, C. H., J. P. HOET AND H. P. MARKS. Proc. Royal Soc. B. **100:** 32, 1926.
BURN, J. H. AND H. H. DALE. J. Physiol. **59:** 164, 1924.
CRUICKSHANK, E. W. H. AND D. L. SHIVASTAVA. This Journal **92:** 144, 1930.
EVANS, C. L., F. GRANDE AND F. Y. HSU. Quart. J. Exper. Physiol. **24:** 347, 1935a.
EVANS, C. L., F. GRANDE, F. Y. HSU, D. H. K. LEE AND A. G. MULDER. Quart. J. Exper. Physiol. **24:** 365, 1935b.
FRIEDEMANN, T. E. AND J. B. GRAESER. J. Biol. Chem. **100:** 291, 1933.
GOOD, C. A., H. KRAMER AND M. SOMOGYI. J. Biol. Chem. **100:** 485, 1933.
HOUSSAY, B. A. AND A. BIASOTTI. Endocrinol. **15:** 511, 1931.
LAVIETES, P. H., J. BOURDILLON AND K. A. KLINGHOFFER. J. Clin. Invest. **15:** 261, 1936.
MANN, F. C. AND T. B. MAGATH. Arch. Int. Med. **31:** 797, 1923.
MARKOWITZ, J., W. M. YATER AND W. H. BURROWS. J. Lab. Clin. Med. **18:** 127, 1933.
RICHARDSON, H. B., E. SHORR AND R. O. LOEBEL. J. Biol. Chem. **86:** 551, 1930.
RUBENSTEIN, B. B. J. Cell. and Comp. Physiol. **2:** 27, 1932.
SOMOGYI, M. J. Biol. Chem. **86:** 655, 1930.
SOSKIN, S. J. Nutrition **3:** 99, 1930.
SOSKIN, S. AND M. D. ALLWEISS. This Journal **110:** 4, 1934a.
SOSKIN, S., M. D. ALLWEISS AND D. J. COHN. This Journal **109:** 155, 1934b.
SOSKIN, S. AND I. A. MIRSKY. This Journal **114:** 106, 1935a.
SOSKIN, S., I. A. MIRSKY, L. M. ZIMMERMAN AND N. CROHN. This Journal **114:** 110, 1935b.
SOSKIN, S., I. A. MIRSKY, L. M. ZIMMERMAN AND R. C. HELLER. This Journal **114:** 648, 1936.
WIERZUCHOWSKI, M. Biochem. Ztschr. **230:** 187, 1931.

THE FUNCTIONS OF THE INTRA-AURAL MUSCLES

HAROLD C. WIGGERS[1]

From the Department of Physiology, Harvard Medical School, Boston, Mass.

Received for publication July 1, 1937

Several conflicting views as to the functions of the stapedius and tensor tympani muscles have appeared in the literature (see Kato, 1913; Lorente de Nó and Harris, 1935). The "protective" theory states that the two muscles contract synergically in response to harmful as well as to innocuous sounds, thereby tending to prevent excessive vibrations from reaching and destroying the delicate sensory apparatus of the inner ear. Contraction of either muscle has been shown to reduce markedly the amplitude of vibration of the drum and ossicular conducting system (Dahmann, 1929). The "accommodation" theory, on the other hand, emphasizes the possibility that these muscles "tune up" the transmission system, thus lowering the threshold for particular frequencies.

The inherent structural obstacles encountered by earlier investigators are now more easily overcome. Electrophysiological methods offer a simplified approach to the function of the middle and inner ear structures. We now recognize that the pattern of sound pressures introduced extra-aurally is microphonically reproduced in the electrical patterns obtained from the cochlea (Davis et al., 1934). It is also possible to detect muscular activity by means of muscular action potentials as well as by visible contraction. We undertook first to determine the effects of contraction of the stapedius and tensor tympani on the other structures of the middle and inner ear, and secondly to measure quantitatively the effect of such activity on the transmission of pure tones across the middle ear.

METHOD. The guinea pig was found most favorable in this investigation. Its middle and inner ear structures closely resemble those of the human except that the apex of the cochlea in the former is freely exposed, offering a favorable point for electrical recording. Furthermore, when these animals are lightly anesthetized with dial-urethane, their intra-aural muscles usually contract spontaneously every few seconds, thus eliminating the necessity of artificial stimulation. The guinea pigs were injected intra-peritoneally with a combination of dial-urethane (Ciba, 0.7 cc. per kilo). Through a postero-lateral approach, a workable opening was made in the osseous bulla, exposing both the round window and the entire

[1] W. T. Porter Fellow of the American Physiological Society.

cochlea from base to apex. The animal was then connected to the sound system by means of a speculum sewed into the external auditory canal.

The apparatus employed for the production of pure tones with variable attenuation, the loud speaker, and the recording instruments were essentially those described by Garceau and Davis (1934). Recent modifications resulting in greater simplicity, reliability, and convenience, which have been introduced by Mr. A. Grass, will be described elsewhere.

The ground electrode was placed upon the neck muscles, the final location being that which introduced the least number of artefacts in the electrogram. The grid electrode was carefully adjusted in contact with either the apex of the cochlea or with the round window promontory, usually the former. The electric potentials were led into a push-pull head amplifier and then through a set of variable filters into a second power amplifier. Thereafter these amplified potential variations were transmitted to a loud speaker and to a cathode-ray oscillograph arranged in parallel. This enabled the investigator to hear and to visualize or photograph concurrently the resulting auditory patterns.

Results. Spontaneous rhythmic contractions of the intra-aural muscles rarely failed to occur when the guinea pigs were anesthetized lightly with dial-urethane. The onset of each contraction coincided with the beginning of an inspiration, but the frequency was usually some submultiple of the frequency of respiration, most often 1:2, 1:3, or 1:4. More rarely ratios of 1:1 and from 1:10 to 1:20 were observed, indicating the degree of variability. The duration of each sequence of contraction and relaxation of both muscles averaged 0.68 second. The strength, abruptness, and frequency of contraction varied considerably with the depth of anesthesia. Inasmuch as the contractions failed to occur when other anesthesias, such as urethane, ether, avertin, and nembutal, were judiciously employed, it is possible that this particular combination or perhaps dial *per se* is essential for the reaction. Reflex contraction to sound was totally absent with this anesthesia. As a matter of fact, continuous introduction of sound only tended to diminish the frequency of these spontaneous contractions and occasionally to inhibit them completely. Upon cessation of the stimulating tone the customary frequency was usually resumed. The injection of a minute spray of chloroform into the osseous bulla usually abolished the intermittent contractions instantaneously. Once inhibited, they never reappeared.

Figure 1a illustrates a typical electrical pattern recorded from the apex of the cochlea during spontaneous contraction of the intra-aural muscles. Its complex nature is due to the superposition of rapid action potentials from the active muscles upon the large slow deflections which result from displacement of the footplate of the stapes and consequent alterations in intracochlear pressures. The slow deflections are always six in number.

Their duration, voltage, and abruptness vary considerably, as indicated in table 1. The action potentials are more distinctly apparent in figure 1b in which the slow deflections are partially filtered out. There are two separate outbursts of potentials, groups A and B. Group A begins immediately previous to the initial slow deflection. Its potentials are smaller and less frequent than those of group B and are summated with the latter which appear at the onset of the second slow deflection. At the termination of the third slow deflection there is an abrupt diminution in the frequency and amplitude of the fast potentials, indicating the dropping

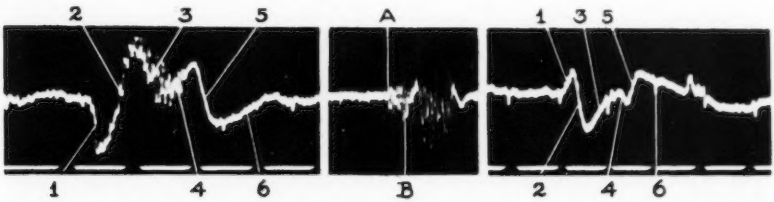


Fig. 1. Left: an electrogram from the apex of the cochlea during spontaneous contraction of the intra-aural muscles. Center: same taken through high pass filter to reduce the slow deflections. Right: a similar electrogram taken from the round window. Time in 0.25 second. See text for identification of the slow deflections *1*, *2*, *3*, *4*, *5* and *6*.

TABLE 1

NUMBER OF DEFLECTION	DURATION OF DEFLECTION	MAGNITUDE OF DEFLECTION	RATE OF POTENTIAL CHANGE
	seconds	*microvolts*	*millivolts per second*
1	0 043	300	7 0
2	0 082	700	8 5
3	0 131	350	2 75
4	0 084	250	3 0
5	0 085	500	5 8

out of one of the groups. The remaining group disappears toward the termination of the fourth slow deflection. For reasons considered below, we attribute group A to the activity of the stapedius, lasting through the first three deflections, and group B to the contraction of the tensor tympani, beginning at the second and terminating near the end of the fourth slow deflection.

Measurement of many records from twenty-eight experiments reveals that the duration of stapedius activity averaged 0.264 second and that of the tensor tympani 0.302 second. The averages of duration, voltage, and rate of change of the first five slow deflections are summarized in

table 1. The sixth is not included because its termination is seldom clearly defined. For comparison, the cochlear electrogram was also recorded from the round window (fig. 1c). Much greater amplification was required to obtain deflections comparable in magnitude to those obtained from the apex. Other differences include *a*, indistinctness of the third and fourth slow deflections; *b*, nearly complete absence of superimposed fast action potentials, and *c*, the opposite polarity of all slow deflections as compared to their homologues in the apical electrogram.

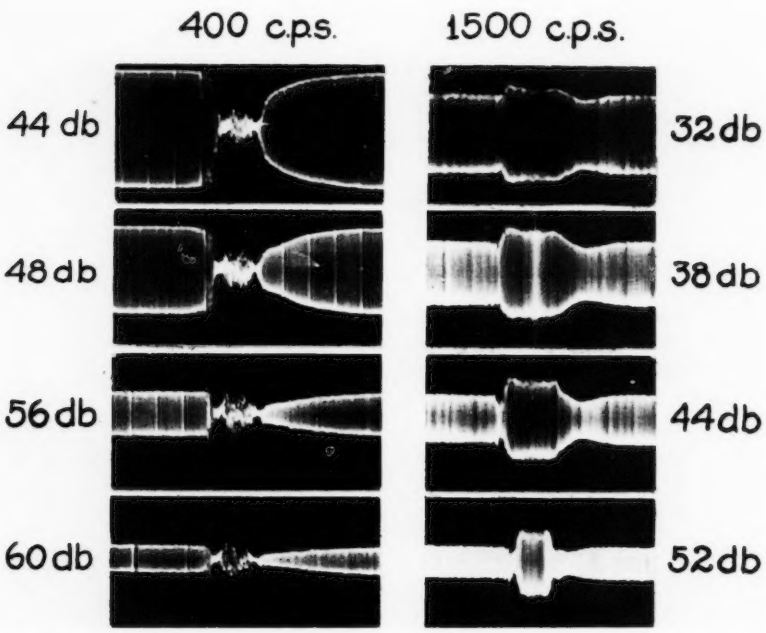


Fig. 2. Electrograms from the apex showing the effect of contraction of the intra-aural muscles on tones of low and medium frequency. The marginal figures give the relative strength of the tones in decibels referred to an arbitrary standard. Speed of film same as in figure 1.

The investigation of the effect of contraction of the intra-aural muscles on sound transmission involved a slightly different procedure. Tones ranging in frequency from 60 to 2500 cycles per second were sounded in the ear of the guinea pig at known intensities from threshold to maximal and the resulting cochlear electrograms were photographed both during rest and during spontaneous contraction of the muscles. Typical electrograms are shown in figure 2. The initial portion of each record taken

before the onset of the spontaneous contraction represents the "rest" response; the middle portion, the "contraction" response. For each frequency, at the various intensities employed, figures for measurements of the average magnitude of the "rest" and "contraction" responses were assembled, converted into logarithmic units of response, and plotted against the intensity of sound in decibels (fig. 3). The continuous lines signify the "rest" response, the broken lines, the "contraction" response over a similar range of intensities.

The effect of the contractions on the transmission of tones of 400 vibrations is opposite to that at 1500 cycles. All graphs for frequencies from 250 to 1000 c.p.s. resembled closely the 400 cycle graph in figure 3. In the latter, the displacement of the "contraction" curve to the right signifies reduced efficiency of transmission during contraction of the intrinsic muscles. The degree of displacement in this range varied inversely with

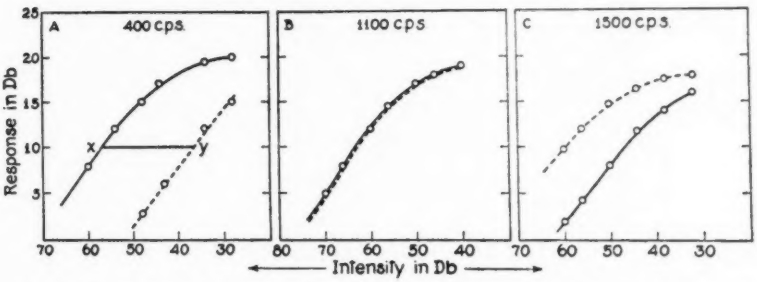


Fig. 3. Explanation in text. Intensities are in decibels below our arbitrary reference level.

the frequency. Graphs for frequencies between 1000 and 1200 vibrations are represented by the 1100 cycle graph in which the "rest" and "contraction" curves are superimposed. There is little effect upon transmission at this range. Graphs for the 1300 to 1800 cycle range are represented by the 1500 cycle graph. The "contraction" curve is herein displaced to the left, indicating augmented efficiency in the transmission system. The peak of augmentation is reached at 1500 cycles.

Whatever the direction or degree of displacement at any frequency, the "rest" and "contraction" curves maintained practically identical slopes over a considerable range of intensities. Any horizontal line *X-Y* (fig. 3a) connecting the linear portions of the two curves measures, therefore, the difference of intensity required to elicit equal electric responses during rest and during contraction. Figures from *X-Y* measurements for graphs of frequencies from 250 to 2500 cycles were employed in the construction of an "audiogram" (fig. 4) which indicates the alteration in threshold pro-

duced by these rhythmic contractions. Due to limitations of the photographic system, to the relatively small voltages at frequencies below 150 cycles, and to the interference encountered from large superimposed action potentials, threshold values below 250 vibrations were obtained by direct observation from the tube face rather than photographically. The differences in threshold are plotted as the open circles in the audiogram.

Discussion. Spontaneous contraction of the intra-aural muscles was first mentioned in 1935 by Stevens et al. The cochlear pattern suggests that each contraction comprises an initial twitch followed immediately by a brief sustained contraction, resembling closely the reflex contraction to sound described by Hallpike (1935).

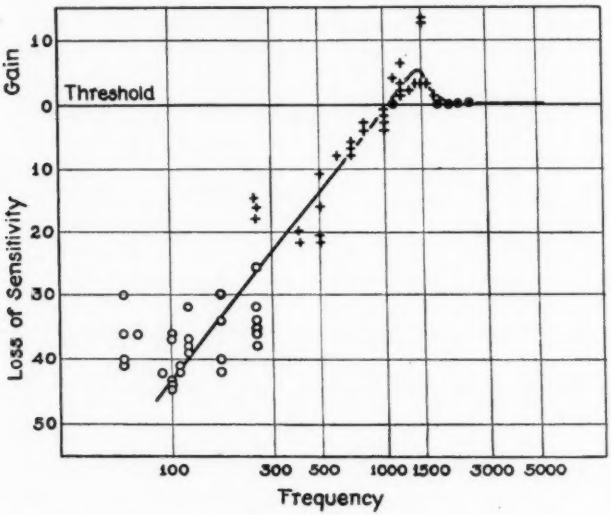


Fig. 4. Audiogram showing the loss or gain of efficiency of sound transmission during contraction of the intra-aural muscles.

The interpretation of the six slow deflections depends entirely upon the explanation of the initial wave, for which there are two possibilities,—contraction of the stapedius or contraction of the tensor tympani. The two groups of action potentials, differing considerably in pattern, clearly suggest that the two muscles do not contract simultaneously. The following considerations show that the stapedius contracts first. The footplate of the stapes is bound to the hyaline cartilage lining the fenestra ovalis by a dense ring of elastic fibers (annular ligament of stapedial footplate), the posterior fibers of which are thicker and shorter than the anterior. In such an arrangement the more pointed anterior end of the footplate is

free to make greater excursions than the posterior (Cunningham, 1902) and the rounded posterior end of the footplate acts as the fulcrum. The stapedius inserts into the posterior aspect of the neck of the stapes, therefore causing an outward displacement of the footplate when it contracts (see fig. 5). Contraction of the tensor tympani causes the opposite (inward) movement of the footplate. The effects on the intra-labyrinthine pressures are opposite in direction. Davis et al. (1935) have shown that inward movement of the stapedial footplate creates a positive change of potential at the apex. An upward deflection in figure 1 denotes such a positive change. But the first slow deflection of the apical electro-

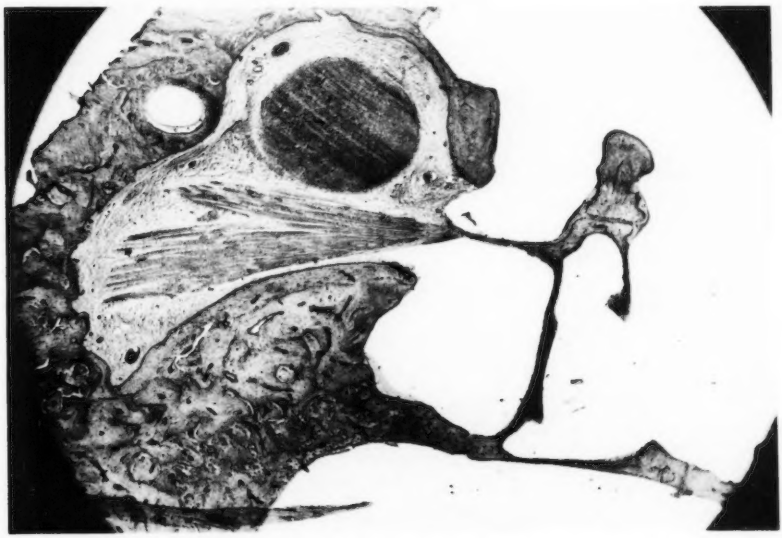


Fig. 5. Microphotograph showing the attachment of stapedius muscle to the posterior aspect of the neck of the stapes. Courtesy of Dr. M. H. Lurie.

gram (fig. 1a and 1b) is negative and therefore represents outward movement of the footplate caused by the stapedius muscle.

When the stapedius has contracted and before normal interscalar relationships can be reëstablished, the tensor tympani contracts, overpowers the sustained stapedius contraction, and forces the footplate inward with the consequent generation of the positive excursion *2*. During the following slow deflection, *3*, the footplate remains inwardly displaced as indicated by the sustained action potentials. Unequal interscalar pressures do not persist, however, for by movement of fluid through the helicotrema, equilibrium is regained as signalled by the gradual return of *3* toward the baseline. This equilibration process is frequently interrupted by relaxa-

tion of the stapedius, indicated by a reduction in amplitude and frequency of action potentials at the onset of deflection *4*. This relaxation of the stapedius tension permits the tensor tympani to thrust the footplate farther inward, thus creating the positive deflection *4*. Shortly thereafter the tensor relaxes, the action potentials disappear and the elastic recoil of the supporting structures shifts the footplate back into its normal position. Pressure is again reduced in the scala vestibuli, creating the large negative deflection *5*. Equilibrium of intrascalar pressures is again established by slow fluid exchange through the helicotrema, as evidenced by the gradual and somewhat indistinct return of *6* to the electrical baseline.

The functions of these muscles can be listed as three—one predominant and two subordinate. The predominant effect of contraction of the intra-aural muscles on sound conduction is the reduction in the efficiency of the system for transmitting tones of low frequency—i.e., tones below 1000 cycles. This loss increases as the frequency decreases until at 100 cycles it approximates 45 decibels. Obviously, therefore, the contraction of the two muscles affords a certain degree of protection against low tones and noises of considerable intensity. This is probably the most important function of these muscles.

It has recently been shown that rather intense harmonics are introduced by the ear itself, especially at low frequencies (Stevens, Newman and Davis, 1937). The characteristics of such harmonics have been studied in animals whose intra-aural muscles had been rendered inactive by chloroform. We suggest that contraction of these intra-aural muscles which reduces the transmission of low tones may eliminate the masking of higher tones by these low tones and their harmonics and thus improve the auditory acuity for higher tones.

The other subordinate function of these muscles is the "tuning up" of the auditory apparatus so that it becomes supersensitive to a few tones at medium frequency. Whereas it is impossible with present information to assign a definite natural period of vibration to the individual elements of the sound-conducting structures, there is evidence for an "over-all" natural period of from 800 to 1500 cycles (v. Békésy, 1936). During contraction of the stapedius and tensor tympani the natural frequency of at least one element of this transmission system is increased, as we should expect from the increase of tension in the system. Consequently tones between 1300 to 1800 cycles are more efficiently transmitted during contraction than during rest, whereas those below 1100 are less efficiently transmitted. Conduction of tones at 1500 c.p.s. are benefited most by this alteration in the period of the system as a whole. Just how efficiently these functions are carried out rests entirely upon the strength of the contractions. The variability of these reflex contractions is shown by the scatter of points in the audiogram in figure 4.

Such intermittent contractions as witnessed in the guinea pig do not occur naturally in humans. It has, however, been established that both intra-aural muscles in the rabbit and guinea pig (Lorente de Nó, 1933) and the stapedius in humans (Luscher, 1929) contract reflexly to sound and that the contractions generally last throughout the exciting tone. In addition, the strength of contraction varies directly with the intensity of the tone and it also varies widely in different animals and individuals. Consequently it is hazardous to define quantitatively the function of these muscles in either man or animal. We do believe, however, that these muscles when reflexly excited in humans must exert protection and accommodation similar to that observed in the guinea pig.

To conclude, let us enumerate the probable functions of the intra-aural muscles. The predominant function is protection against low tones and noises; the subordinate functions are the improvement of auditory acuity as a result of 1, a change in the natural frequency of at least one element of the transmission system, and 2, the elimination of the masking effects of low tones and their harmonics.

SUMMARY

The intra-aural muscles of a guinea pig under dial-urethane anesthesia contract spontaneously from time to time. The electric variations accompanying the intralabyrinthine pressure changes induced thereby and the action potentials accompanying such activity indicate that the stapedius contracts prior to the tensor tympani by approximately fifty milliseconds but that the contraction of the latter muscle overpowers and outlasts that of the former.

Electrograms obtained during tonal stimulation while the muscles are contracted revealed that these muscles when active can significantly reduce the efficiency of transmission for low tones below 1000 cycles and that the lower the tone, the greater the reduction. Transmission of medium frequency tones (1300 to 1800 cycles) is noticeably enhanced by contraction of these muscles, the greatest amount of improvement being observed at 1500 cycles per second. Transmission for tones close to 1100 and those above 2000 cycles are unaffected by contraction of the tensor tympani and stapedius muscles. These observations give more support to the protective theory than to the accommodative theory of the function of the intra-aural muscles.

I wish to acknowledge my indebtedness to Dr. Hallowell Davis for his valuable criticisms and guidance in the prosecution of this investigation.

REFERENCES

v. Békésy, G. Akustiche Ztschr. **1**: 13, 1936.

Cunningham, D. J. Textbook of anatomy. MacMillan Company, New York, pp. 712–713, 1902.

DAHMANN, H. Ztschr. f. Hals- Nasen- und Ohrenheilk. **24**: 462, 1929.
DAVIS, H., A. J. DERBYSHIRE, M. H. LURIE AND L. J. SAUL. This Journal **107**: 311, 1934.
GARCEAU, E. L. AND H. DAVIS. This Journal **107**: 305, 1934.
HALLPIKE, C. S. J. Laryngology **50**: 1935.
KATO, T. Pflüger's Arch. **150**: 569, 1913.
LORENTE DE NÓ, R. Trans. Amer. Laryng., Rhinol. and Otological Soc., 1933. The Laryngoscope **45**: August, 1935.
LORENTE DE NÓ, R. AND A. S. HARRIS. The Laryngoscope **43**: 315, 1933.
LUSCHER. Ztschr. Hals usw. Heilk. **23**: 105, 1929.
STEVENS, S. S., H. DAVIS AND M. H. LURIE. J. Gen. Psychol. **13**: 297, 1935.
STEVENS, S. S. AND E. B. NEWMAN. Proc. Nat. Acad. Sci. **22**: 668, 1936.
STEVENS, S. S., E. B. NEWMAN AND H. DAVIS. J. Acoust. Soc. (in press), 1937.

A STUDY OF DENERVATED MAMMALIAN SKELETAL MUSCLE

A. ROSENBLUETH AND J. V. LUCO[1]

From the Department of Physiology in the Harvard Medical School

Received for publication July 1, 1937

In previous reports from this Laboratory (Rosenblueth, Lindsley and Morison, 1936; Rosenblueth and Morison, 1937a, b) characteristic changes in the responses of skeletal muscle under various experimental conditions have been attributed to events occurring at the neuromuscular junction. Although in these experiments several controls were made which justified this localization of the phenomena reported, it was deemed important to test denervated muscles in similar experimental conditions, with the purpose of further separating neuromuscular from muscular events. A study of denervated muscles afforded also the opportunity of testing certain implications and corollaries of the theory of chemical mediation of motor nerve impulses by acetylcholine (cf. Brown, Dale and Feldberg, 1936).

METHOD. Cats were used, under dial (Ciba, 0.7 to 0.8 cc. per kgm. intraperitoneally) anesthesia. The muscles studied were the gastrocnemius-soleus, the quadriceps, the sartorius, and the muscles of the tongue and face. They were denervated by aseptic section of a sciatic, femoral, hypoglossal or facial nerve 6 to 40 days before the experiments.

When the leg muscles were employed the limb was fixed by means of drills in the tibia or femur. The tendons of quadriceps or gastrocnemius-soleus were freed and attached to the myograph after cutting away the regions of bone where they insert. Sartorius was not used as a whole, but a small bundle was separated, leaving its blood supply intact, and cut from its tibial attachment.

For the study of the facial and tongue muscles the head was held by a Czermak clamp. The only muscle of the head dissected and studied mechanically was the posterior auricular, which may be readily separated and cut at the ear. In other facial muscles (auriculo-labialis, orbicularis oculi and canine) and tongue muscles only electrical responses were studied. No dissection was made. The tongue was merely pulled out by a clamp attached to the tip. The contractions in these cases were physiological—heterometric and heterotonic.

The mechanical records were usually tension records. Kymograph inscriptions were obtained by making the muscles pull the short arm of a

[1] Fellow of the Catholic University of Chile.

lever against stiff rubber bands; the magnification was about 7-fold. Frictionless torsion-spring optic myographs were also occasionally employed. Isotonic recording was only used for the posterior auricular and sartorius muscles; a moderately weighted lever furnished kymograph tracings.

The electric responses were led to the amplifier usually by concentric needle electrodes inserted into the muscles. Occasionally bipolar silver or platinum wire electrodes, applied to the surface of dissected strands of muscles, were also employed. The amplifier and recording outfit were the same as in the study by Rosenblueth and Morison (1937a).

For direct stimulation of the muscles the usual procedure was to insert silver-silver chloride or steel needles into their bodies and tendons. When the electric responses to direct stimulation were observed the stimulating electrodes were silver wires with an interpolar distance of from 3 to 8 mm., applied to the surface of the dissected strands and shielded by rubber from

Fig. 1. Action of eserine on the spontaneous electrical activity of a denervated tongue. Right hypoglossal nerve cut 9 days previously. Dial; atropine (1 mgm. per kgm.); adrenals ligated; left hypoglossal cut. Concentric electrodes in right side of tongue. Time: 10 msec. intervals. A, before eserine; B, thirty seconds after injecting eserine (0.5 mgm. per kgm.); C, one minute later.

surrounding tissues. Similar electrodes were used for stimulation of nerves.

The electrical stimuli differed for the several series of observations. Induction shocks from a Harvard coil were sometimes employed, either for single pulse or for repetitive activation. Condenser discharges of various capacities were also used; their frequency was controlled by a mechanical interrupter. Rectangular waves from a multivibrator circuit were sometimes applied. Finally, for the study of the electric responses, the electrical stimuli were amplified waves from a photoelectric cell rendered diphasic by passage through a transformer; single waves were obtained by interrupting the source of light by means of an ordinary Kodak shutter or a Graflex focal-plane shutter, and repetitive stimulation resulted from rotating disks cut by slits of adequate number and dimensions and driven by a synchronous motor giving 1 or 30 revolutions per second.

A tracheal cannula was inserted for artificial respiration, when necessary.

The adrenals were routinely ligated in all the experiments in which acetylcholine was injected; atropine (1 mgm. per kgm.) was also always given in these experiments and in those in which eserine or prostigmin was employed. All injections were made intravenously.

RESULTS. A. *The spontaneous electrical activity.* All the denervated muscles studied showed a continuous irregular, asynchronous series of spike potentials (cf. figs. 1, 5 and 11). The earliest observations were made 5 days and the latest 40 days after section of the motor nerves. In general the spontaneous activity was greatest 10 to 30 days after the section. Although the time course of the development of this activity was not accurately traced it seems probable that it is correlated with the

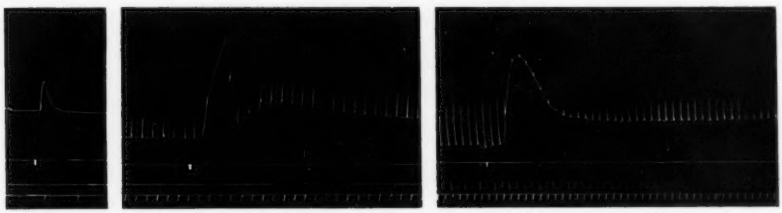


Fig. 2 Fig. 3 Fig. 4

Fig. 2. Response of denervated (11 days) gastrocnemius-soleus to eserine (1 mgm. per kgm.). Dial; atropine (1 mgm. per kgm.); adrenals ligated.

Fig. 3. Lack of refractoriness of muscle to direct stimulation during a contracture elicited by acetylcholine. Gastrocnemius-soleus denervated 7 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. Single condenser discharges applied regularly as shown by the twitches. At signal 0.5 mgm. acetylcholine injected intravenously. Time: 5 sec. intervals.

Fig. 4. Independence of the contracture and the refractoriness resulting from acetylcholine after eserine. Gastrocnemius-soleus denervated 40 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated; eserine (1 mgm. per kgm.). The middle signal indicates make and break single induction shocks applied directly throughout. At upper signal acetylcholine (50 γ) injected intravenously. Time: 5 sec. intervals.

progress of degeneration of the muscles, appearing with the onset of degeneration and disappearing only with complete fibrosis. Such activity is not encountered even 10 hours after section of the motor nerves of normal muscles, in acute experiments.

Injections of atropine (1 mgm. per kgm.) did not significantly modify the spontaneous activity. Curare, in doses 2 or 3 times greater than was necessary to paralyze the respiratory movements, led usually to only a slight reduction of the spontaneous spikes. Eserine (0.5 to 2 mgm. per kgm.) or prostigmin (0.2 to 1 mgm. per kgm.) produced invariably a transient increase of their number and magnitude (fig. 1). This increase of electrical activity was attended by a mechanical contraction (fig. 2).

Direct stimulation of the muscles, either by single shocks or repetitively led frequently to prolonged contraction-remainders succeeding the twitche or tetani. These contraction-remainders appeared electrically as a marked increase in the amplitude and frequency of the spike potentials in the spontaneous background.

B. *Responses to acetylcholine.* The sensitivity of denervated mammalian muscles to acetylcholine is well known (Frank, Nothmann and Hirsch-Kaufmann, 1922). Schäffer and Licht (1926) studied the electrical phenomena attending the contractions evoked by the drug and observed

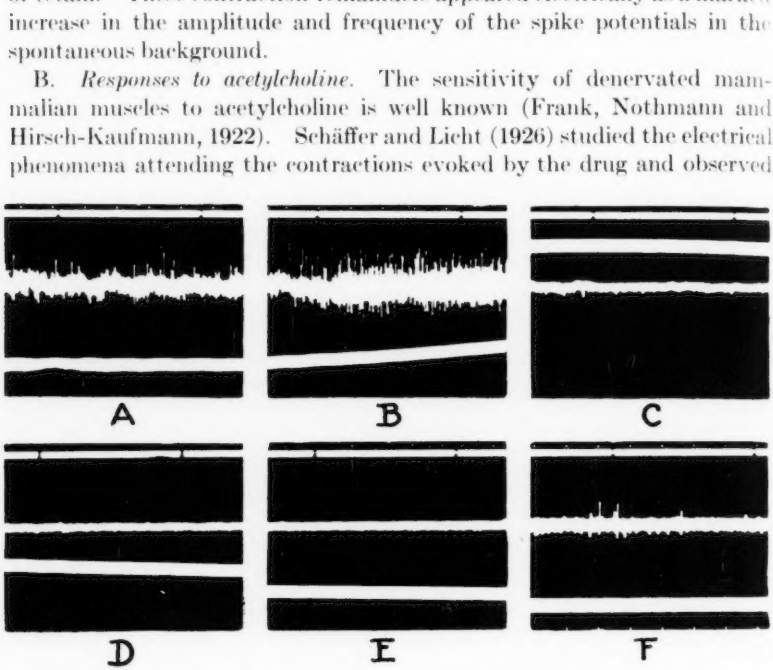


Fig. 5. Contraction and contracture elicited by acetylcholine. Gastrocnemius denervated 15 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. The irregular tracing in the middle of the film is the electrogram (concentric electrodes) and the smooth line beginning at the bottom of the record is the isometric (frictionless torsion-spring optic myograph) mechanogram. Time: 1 sec. intervals. A, background 2 sec. after injection of acetylcholine (1 mgm.); B, contraction, 3 sec. later; C, peak of the contracture, 5 sec. later (mechanogram above); D, relaxation, 10 sec. later; E, further relaxation, 12 sec. later; F, one minute later.

only slow, smooth deflections of the string galvanometer, coincident with the mechanical excursions. Recently, however, Brown (1937b) has reported typical action potentials on injecting small doses of acetylcholine intra-arterially; with larger doses an initial outburst of spike potentials was followed by a period of silence of the electrogram, during which even the spontaneous electrical activity was absent; the mechanical records showed a "quick" contraction, associated with the action potentials, clearly distinct from a slow succeeding contraction during the silent period of the electrograms.

Our observations are in complete accord with those of Brown. The mechanical responses to acetylcholine can be divided into two components: a contraction, attended by spike potentials, and a contracture, in which no spike potentials may be detected (fig. 5). The amplifier used in this study does not allow very slow changes of potential to be recorded. Indeed, our interest being mainly centered on the spike potentials, small-capacity condensers were commonly used to eliminate slower changes which would render the records confusing. For this reason the present observations do not furnish any basis for confirmation of Schäffer and Licht's (1926) report of slow changes of potential simultaneous with the changes of tension.

Brown's (1937b) observation that the appearance of a contracture requires larger doses of acetylcholine than does the contraction was also confirmed. There are other factors of importance, moreover, in determining the relative proportions of contraction and contracture in the

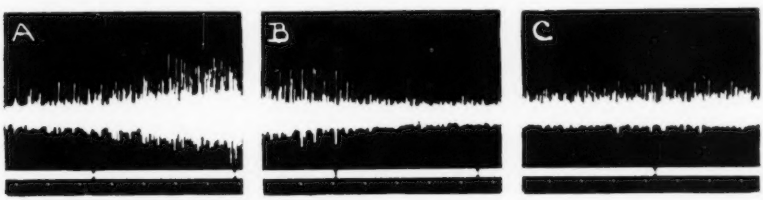


Fig. 6. Initial and delayed increase of electrical activity in response to acetylcholine. Anterior auricular muscle denervated 14 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. Time: 1 sec. intervals. A, beginning of response, 10 sec. after injecting acetylcholine (1 mgm.); B, depressed activity, 6 sec. later; C, delayed increased activity, 3 sec. later.

responses recorded. First may be mentioned the number of days elapsed after severance of the motor nerve. If the observations are made 5 or 6 days after the section even large doses of acetylcholine (up to several mgm.) will elicit mainly or exclusively contractions. Later (8 to 12 days) the contractures become very apparent with adequate doses, but their magnitude is approximately similar to that of the contractions (ratios of 0.5 to 4). When the muscles have been denervated longer (15 to 30 days) only small contractions are usually elicitable, while large doses of acetylcholine may evoke quite large contractures. It appears, therefore, that the sensitivity of the contracture response to acetylcholine develops more slowly than the contraction response and that the increase of the contractures occurs simultaneously with a relative curtailment of the contractions.

The electrical records obtained during the responses to acetylcholine revealed occasionally several interesting features. Thus, in some instances, an initial large burst of activity was followed by a period of

relative quiescence, in turn followed by a delayed increase of electrical activity (fig. 6; cf. Brown, 1937b). In favorable instances, when the electrogram of a single muscle fiber could be recognized and followed, its cessation after relatively large doses of acetylcholine was sudden—without any sign of slowing in the frequency of discharge (fig. 7).

After injections of eserine or prostigmin much smaller doses of acetylcholine injected intravenously are required to elicit responses in the muscles than before administration of these drugs. Prostigmin is 3 to 10 times more effective than eserine—i.e., similar potentiations of the actions of acetylcholine occurred with a given dose (mgm. per kgm.) of prostigmin as with 3 to 10 times that dose of eserine.

The action of acetylcholine was further modified after eserine or prostigmin as regards the relative proportion of the contractions and contractures recorded. In general it may be stated that eserine tended to curtail the contractions and enhance the contractures. Although the

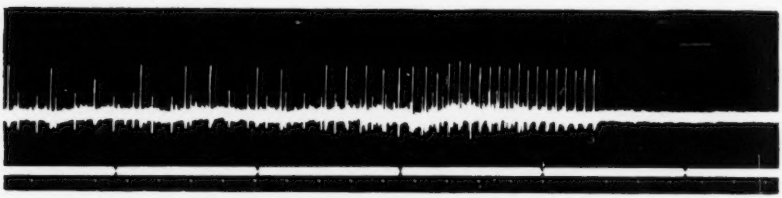


Fig. 7. Sudden cessation of the spike potentials of a fiber responding to acetylcholine. Gastrocnemius denervated 7 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. Injection of acetylcholine (0.2 mgm.) 8 sec. before the record. Time: 1 sec. intervals.

contractures promptly declined from the maximum, much as in non-eserinized muscles, very prolonged contracture-remainders (5 to 60 min. or more) were the rule after eserine, even with moderate doses of acetylcholine (see fig. 4).

Injections of curare, even in doses insufficient to produce paralysis of the respiratory movements, markedly reduced the effects of subsequent injections of acetylcholine. The threshold for both the contractions and the contractures was raised—i.e., much larger amounts of acetylcholine were necessary to elicit the responses (Brown, 1937b). The ability of acetylcholine to silence all the electrical activity of the muscles (cf. fig. 5) was also depressed. This influence of curare on the responses to acetylcholine occurred both with and without previous injections of eserine.

When a series of injections of a given small dose of acetylcholine was made at regular intervals (e.g., 1 or 2 min.) a uniform series of responses occurred. Direct tetanization of the muscle for 5 to 15 seconds led to a

large transient increase of the responses to acetylcholine (fig. 8). The greatest responses occurred immediately after the tetanus; thereafter a slow (5 to 10 min.) subsidence of the potentiation was apparent. The phenomenon is quite reversible, it could be repeated several times in the same muscle.

The post-tetanic increment of the responses to acetylcholine occurred mainly or exclusively by an increment of the contracture as opposed to the contraction. Indeed, the contractions seemed slightly less after than before the tetanus. This observation was made with both mechanical and electrical records.

Since acetylcholine evokes repetitive discharges from the muscles—i.e., tetani—and since tetani result in an increase of the responses to acetylcholine, it appeared plausible that adequate doses of acetylcholine injected at adequate intervals should show increasing effects, each new injection

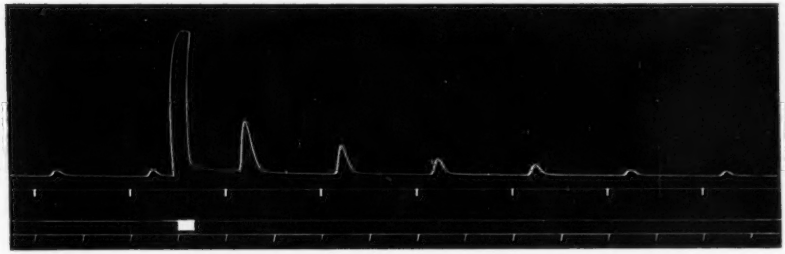


Fig. 8. Post-tetanic increase of the responses to acetylcholine. Gastrocnemius-soleus denervated 11 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. At upper signals 0.25 mgm. acetylcholine injected intravenously. At middle signal direct stimulation of the muscle. Time: 30 sec. intervals.

being sensitized by the tetanus resulting from the preceding dose. This expectation was readily confirmed. Whereas small doses of acetylcholine injected every 1 or 2 minutes yield uniform responses, slightly larger doses may lead to successively increasing effects, until an equilibrium is reached.

C. *Responses to single shocks.* The influence of injections of acetylcholine on the mechanical twitches in response to single shocks depends on various factors: the dose of acetylcholine, the duration and intensity of the shocks, the presence or absence of eserine.

Subliminal doses of acetylcholine were found to be without any influence on the responses to shocks of various intensities and durations. Small doses of acetylcholine, leading only to small contractions, without any contractures, were likewise without any significant action on the responses to single shocks.

Large doses of acetylcholine have complex effects. During the phase of

contraction there is no evidence of decreased excitability; although the superimposed twitches are as a rule smaller than those of the resting muscle, probably because some of the units are refractory, every shock evokes a twitch (fig. 3).

During the phase of contracture the muscle may be refractory to direct stimulation (cf. Gasser and Dale, 1926; Brown, 1937b). That the refractoriness is not necessarily correlated with the contracture is shown, however, by the three following facts: 1, it is possible to obtain refractoriness from acetylcholine without a contracture (Gasser and Dale, 1926); 2, the recovery from refractoriness may occur independently of the subsidence of a contracture (fig. 4); 3, contractures may occur without apparent refractoriness (fig. 3). Eserine or prostigmin greatly increase the period of

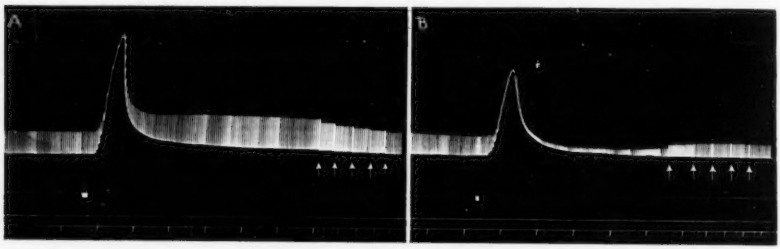


Fig. 9. Influence of the duration of the shocks on the post-acetylcholine submaximal twitches. Gastrocnemius-soleus denervated 9 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. At signals 10 mgm. acetylcholine injected intravenously. Rectangular single shocks from a multivibrator applied throughout at the frequency indicated by the twitches. Time: 30 sec. intervals. At the points signalled by the arrows the kymograph was stopped for 1 minute. A, length of single shocks: 2 msec., post-acetylcholine increase of the twitches; B, length of single shocks: 100 msec., post-acetylcholine decrease of the twitches.

refractoriness, apparently more than they do that of the contractures; indeed, 0.2 to 1 mgm. of acetylcholine, injected after eserine, may render the muscle refractory for 30 to 60 minutes.

When a dose of acetylcholine is injected (preferably without eserine), which leads to a large contraction and only a slight contracture, it may have marked effects on the submaximal twitches elicited by a given stimulus *after* the response to acetylcholine has entirely subsided. If short shocks (condenser capacities of 0.05 to 0.1μF, resistance 5000ω; or d.c. pulses of 0.5 to 2 msec.) are delivered regularly, a transient (several minutes) post-acetylcholine increase of the twitches is the rule (fig. 9A). If long shocks (capacities: 5 to 6μF; d.c.: 50 to 100 msec.) are used there may be no change after acetylcholine, or even a transient decrease of the

twitches (fig. 9B). When the test-shocks were supramaximal, no post-acetylcholine changes were detected.

Eserine or prostigmin usually reduce slightly the magnitude of the twitches elicited by a given submaximal stimulus. A potentiation, with repetitive electric responses, such as occurs in normal muscles stimulated indirectly (Brown, Dale and Feldberg, 1936), was never observed.

The influence of curare on the responses to single shocks was studied in three animals by constructing strength-duration curves for different levels of response before and after injection of a dose of curare 2 to 4 times larger than was necessary to paralyze the respiratory movements. In two of these cats there was no significant change of the strength-duration curves of the denervated gastrocnemius after curare. In the third animal the excitability of the muscle was markedly increased by the drug, the rheobase was considerably lower, while the chronaxie was only slightly shorter than before administration of curare.

The submaximal twitches to given stimuli showed a transient increase after a tetanic stimulation (5 to 15 sec.) when the single shocks were short. Long shocks did not reveal any post-tetanic increments.

The electric responses of the muscles to single shocks varied always parallel to the mechanical responses—i.e., increased or decreased together with the twitches. Thus, the term refractory was used to denote the lack of responses of the muscles to electrical stimulation after injections of large doses of acetylcholine, because both electric and mechanical responses were absent at this period.

D. *Responses to tetanic stimulation.* The quadriceps and gastrocnemius muscles were found capable of yielding well-sustained responses for 30 to 60 sec. up to frequencies of about 80 per sec. With higher frequencies (100 or more per sec.) the responses subsided during stimulation, the more rapidly the higher the frequency.

Injections of eserine or prostigmin led to a slight depression of the tetanic responses, both in magnitude and in sustaining ability—i.e., at 60 per sec. the tetani were frequently not fully sustained after eserine. In no instance, however, was a prompt decline (Wedensky inhibition) observed with frequencies of from 30 to 90 per sec., such as occurs in innervated muscles stimulated indirectly (Cowan, 1936; Briscoe, 1936). In no instance was a delayed rise of tension observed, after an initial decline, such as occurs typically in eserinized muscles activated indirectly at high frequencies (Cowan, 1936).

Injections of curare (up to 4 times the dose necessary for paralysis of respiration) were practically without influence on the tetanic responses of the muscles. In no case was Wedensky inhibition observed, such as is induced by the drug in innervated muscles activated indirectly at slow frequencies (Hofmann, 1903).

When tetanic stimulation was applied for long periods (10 or more min.) at a frequency, e.g., of 60 per sec., the tension slowly declined until practically complete fatigue. The electric responses declined in a quite parallel fashion until complete disappearance.

E. *The Philipeaux-Vulpian and Rogowicz phenomena.* It is well known that stimulation of the chorda tympani after section and degeneration of the hypoglossal nerve leads to a contraction of the denervated muscles of the tongue (Philipeaux and Vulpian, 1863). Similarly, stimulation of the cervical sympathetic leads to contraction of the denervated facial muscles (Rogowicz, 1885). Schäffer and Licht (1926), studying the tongue muscles, could find only smooth prolonged electrograms, devoid of spikes, during the contractions occurring on stimulation of the lingual nerve. These negative results are probably due to the lack of speed and sensitivity of the string galvanometer they used, in view of the present observations.

Stimulation of the cervical sympathetic for 10 to 30 sec. at frequencies of from 18 to 60 per sec. led usually to an initial (2 or 3 sec.) decrease of the electrical spontaneous background of the denervated facial muscles (posterior auricular, orbicularis oculi, canine, and auriculo-labialis). This initial depression was followed by a striking increase of the electrical activity of the muscles, surpassing markedly the original spontaneous background. The increased activity slowly (5 or more sec.) subsided to the original level at the end of stimulation. Figure 10 is a typical instance.

For the study of the tongue the intact hypoglossal on the non-operated side and the two facial nerves were cut. The lingual nerve was also cut on both sides before it crosses the submaxillary ducts and stimulating electrodes were applied peripherally. Before proceeding to the account of the results of stimulation of the lingual on the denervated muscles, an anomaly, which may be a source of error, should be pointed out.

In two of the eight animals studied with a hypoglossal nerve previously cut, stimulation of the lingual led to mechanical responses entirely similar to those which stimulation of the hypoglossal elicits in normal tongues—i.e., prompt contractions subsiding rapidly at the end of stimulation. Analogous responses occurred in one out of three innervated tongues (hypoglossals cut acutely) in which the lingual was similarly stimulated. Electrical responses quite similar to normal motor electrograms—i.e., single spikes with a latency of about 10 msec. for every shock applied—were recorded in these animals when the concentric lead-off electrodes were in certain positions in the tongues, not in others. It appears probable that in some cats aberrant motor fibers from the facial nerve may reach the tongue via the chorda tympani and that these motor fibers were responsible for the responses just described.

With these records discarded as atypical, repetitive stimulation of the lingual nerve led to a prompt increase of the electrical activity in the

spontaneous background. The peak of this increase reached usually a maximum about 20 sec. after the beginning of stimulation and then subsided, although the stimulus was continued (fig. 11).

Stimulation of the cervical sympathetic did not elicit any changes in the tongue muscles. Single shocks applied to either the cervical sympathetic

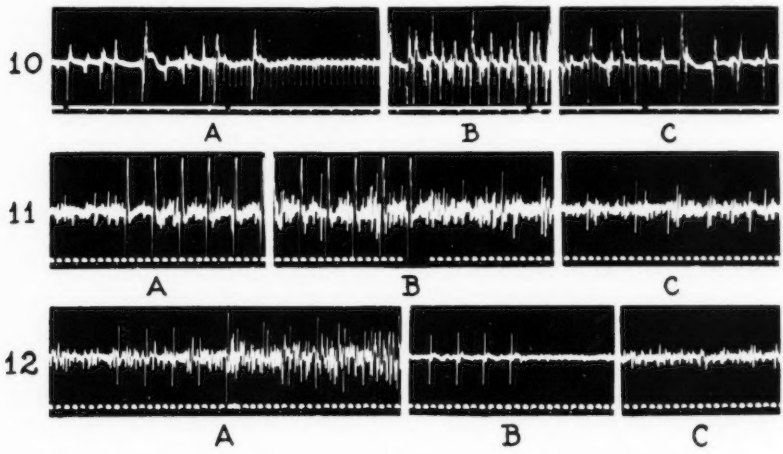


Fig. 10. The Rogowicz phenomenon. Posterior auricular muscle denervated 13 days. Dial; atropine (1 mgm. per kgm.); eserine (1 mgm. per kgm.). Stimulation of the cervical sympathetic at the rate of 30 per sec. for 10 sec. Concentric electrodes. Time: 1 sec. intervals. The fine descending lines are the stimulus artifacts. A, beginning of stimulation; B, three seconds later; C, end of stimulation.

Fig. 11. The Philipeaux-Vulpian phenomenon (without eserine). Right hypoglossal cut 6 days previously. Dial; atropine (1 mgm. per kgm.); left hypoglossal cut. Concentric electrodes in right side of tongue. Right lingual nerve stimulated 30 sec. at the rate of 30 per sec., as shown by the large stimulus artifacts. Time: 10 msec. intervals. A, beginning of stimulation; B, end of stimulation; C, ten seconds later.

Fig. 12. The Philipeaux-Vulpian phenomenon (after eserine). Right hypoglossal cut 7 days previously. Dial; atropine (1 mgm. per kgm.); adrenals ligated; left hypoglossal and both facial and lingual nerves cut. Eserine (3 mgm. per kgm.). Stimulation of the right lingual nerve for 10 sec. at the rate of 30 per sec., as shown by the diphasic stimulus artifacts. Time: 10 msec.; the slight irregularities in the time scale were due to momentary jams in the gears of the camera. A, beginning of stimulation; B, end of stimulation; C, one minute later.

or the lingual nerve failed to produce any recordable changes on the non-eserinized facial or tongue muscles, respectively.

Injections of eserine or prostigmin led to augmented, mechanical responses of the facial (Euler and Gaddum, 1931) and tongue muscles. Electrically, the corresponding changes were manifested by a greater ease

of obtaining more marked initial augmentation and subsequent depression of the electrical activity. Figure 12 illustrates typical results of repetitive stimulation of the lingual nerve after eserine.

Single shocks applied to the cervical sympathetic failed to elicit any significant change in the electrograms of the denervated facial muscles, even after eserine. Single shocks applied to the lingual nerve, on the other hand, resulted, in two out of four animals tested in this regard, in the characteristic responses of the eserinized denervated tongue muscles illustrated in figure 13. After a latency of about 60 msec. a burst of spike potentials is succeeded by a decreased electrical activity, showing fewer waves than the original background; this quiet period is followed

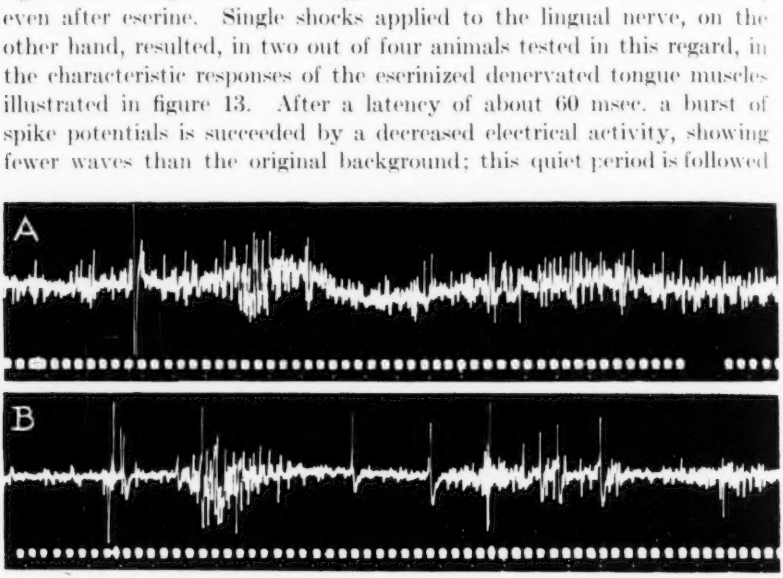


Fig. 13. The Philipeaux-Vulpian phenomenon in response to single nerve volleys (after eserine). Right hypoglossal cut 7 days previously. Dial; atropine (1 mgm. per kgm.); eserine (1 mgm. per kgm.); left hypoglossal cut. Single discharges from a photoelectric cell briefly illuminated applied to the right lingual nerve, as shown by the large diphasic stimulus artifact. Time (short intervals): 10 msec. A and B, two different positions of the concentric electrodes in the right side of the tongue.

shortly by renewed enhanced deflections which subside slowly (5 to 15 sec.) to the background level.

Discussion. The continuous electrical (cf. fig. 1) and mechanical fibrillary activity of denervated skeletal muscles is a puzzling phenomenon. It is probably not due to a continuous local production of acetylcholine acting on the sensitized muscle fibers, for curare and eserine, which modify deeply the action of acetylcholine (section B), affect only slightly the spontaneous background (fig 1; section A). It is further improbable that acetylcholine is involved in the phenomenon, since Dale, Feldberg and Vogt (1936) could detect no release of acetylcholine on direct stimulation of denervated gastrocnemii. An analogy to the continuous activity of the

degenerating muscles is furnished by the continuous discharges of degenerating severed nerves in the tail of fishes, which Parker (1934) demonstrated. Future studies may reveal whether the analogy is meaningful.

The results reported in section B (figs. 5, 6 and 7) give support to the view that acetylcholine is capable of setting up typical contractions of skeletal muscles, preceded by propagated disturbances (Brown, 1937a and b). These results emphasize further that acetylcholine has other effects on denervated mammalian muscles—contractures not attended by spike potentials (fig 5; Schäffer and Licht, 1926; Brown, 1937b) and a prolonged depression of the electrical excitability until the muscles appear entirely refractory to direct electrical stimulation (Gasser and Dale, 1926).

In systematizing these diverse effects of acetylcholine it is important to find out whether each may occur independently of the others or whether they are mutually related. Since contractions may occur without any contractures, and since, furthermore, the muscles do not reveal any refractoriness either during or after the contractions, other than that which can be reasonably attributed to the refractory period of the elements activated during the contraction (fig. 3), it is quite clear that this first effect of acetylcholine is independent of the others. In consonance with Langley's (1905) study of the action of nicotine it appears probable that acetylcholine acts at the motor plates in setting up propagated disturbances similar to those set up by motor nerve impulses in innervated muscles. The sudden cessation of electrical activity of the recording unit in figure 7 can best be explained by the paralytic action which high doses of acetylcholine have on the motor plate (cf. Rosenblueth, Lindsley and Morison, 1936). A similar explanation may apply for the intermediate period of relative quiescence in the response to acetylcholine illustrated in figure 6.

Whether the contractures and the refractoriness of the muscles are dependent or independent phenomena is at present an open question. The facts enumerated on p. 788 lend weight, however, to the inference that they may occur independently. In normal muscles activated indirectly it is easy to block the transmission of nerve impulses by acetylcholine with little or no concomitant contracture (Rosenblueth, Lindsley and Morison, 1936).

The view developed, that the refractoriness of the muscles is independent of the contractures, is theoretically justifiable, as follows. The contractures reveal a direct effect on the contractile system—i.e., not an indirect consequence of propagated disturbances. The refractoriness to electrical stimulation, on the other hand, according to the current membrane theory, denotes probably an interference with the conducting mechanism of the muscles. The independence of the two effects would result then from their being related to different systems in the muscle, and on the independence of these systems (cf. Gasser and Dale, 1926).

Other results of injections of acetylcholine, namely, those designated as post-acetylcholine effects (fig. 9, p. 788), may be explicable as resulting from the tetani set up by the drug—i.e., they may be grouped with the post-tetanic effects (fig. 8, p. 787). These post-tetanic effects, especially the increments of the twitches, are quite different from those which occur in innervated muscles activated indirectly (Schiff, 1858; cf. Rosenblueth and Morison, 1937a). The present increments apparently denote merely a change of electrical excitability of the muscle fibers—i.e., the result is due to spatial summation—whereas the indirect post-tetanic increments, which occur when maximal shocks are applied to the nerves, reveal in all probability repetitive discharges at the neuromuscular junction (Rosenblueth and Morison, 1937a, b)—i.e., temporal summation.

The study of the effects of curare (p. 786) furnishes direct evidence for Dale's (cf. Briscoe, 1936) suggestion that curare raises the thresholds of the muscle to acetylcholine, both the excitatory and the paralytic. The observations on the changes of electrical excitability of the muscles after curare (p. 789), although only few in number, lend no support to Lapicque's (1926) theory of curarization. The main point to be emphasized here is that great changes in the threshold to acetylcholine occur after doses of curare which either do not modify or even increase the electrical excitability of the muscles.

The effects of eserine or prostigmin appear to be complex. The reduction of the threshold to acetylcholine administered intravenously can be readily explained by the well-known anti-esterase activity of these drugs. The results suggest, however, that eserine has some direct effects on the muscles, which are not related with its ability to protect acetylcholine from destruction. Thus, the action of eserine on the spontaneous electrical activity (fig. 1) and the corresponding contraction (fig. 2) are probably not due to protection of acetylcholine (see p. 792). This contraction was not observed by Langley and Kato (1915), presumably because of its slight magnitude and rapid evanescence, but it occurred consistently in the present experiments. More enduring are the depressing effects of eserine or prostigmin on the electrical excitability of the muscles, as evidenced by the decreased and less sustained responses which occur up to one hour after injections of moderate doses of the drugs (pp. 788, 789). This decreased excitability is probably also due to a direct action of eserine on the muscles, not to inactivation of the cholinesterase.

The present results furnish controls for the localization of the following phenomena at the neuromuscular junction (cf. Rosenblueth and Morison, 1937a): Wedensky inhibition after eserine or curare; the delayed rise in tension of normal or eserinized muscles stimulated indirectly at high frequencies; the post-tetanic increase of maximal twitches of normal, eserinized or fatigued muscles activated indirectly; and the post-tetanic

decurarization of Boyd (1932). These phenomena are absent in denervated muscles stimulated directly (sections C and D).

The bearing of the results on the several theories advanced to explain the transmission of motor nerve impulses to skeletal muscle may now be discussed. The action of curare, used by Lapicque (1926) to support the theory of an electrical transmission, is in view of the present evidence a strong argument against such electrical transmission. Thus, the influence of curare on the electrical excitability of muscle is either negligible or else it consists in an enhancement of this excitability (p. 789; cf. Watts, 1924; Grundfest, 1932; Rushton, 1933). On the other hand, curare raises considerably the threshold of the muscle to acetylcholine (p. 786). The block of the motor nerve impulses by curare is therefore readily explained if acetylcholine is the chemical mediator of these nerve impulses (Brown, Dale and Feldberg, 1936); it would remain unexplained if the muscle were activated by the action potential of the nerve, as the theory of electrical transmission postulates.

The effects of eserine constitute also an important argument against the theory of electrical transmission. Eserine decreases the electrical excitability of muscles (p. 789); on the other hand, it protects acetylcholine from destruction by cholinesterase. The increase by eserine of the muscular responses to single nerve volleys (Brown, Dale and Feldberg, 1936) remains, therefore, unexplained by the electrical theory of transmission, whereas it is readily covered by the chemical theory.

The strongest support which the present study contributes to the theory of humoral activation of skeletal muscle is furnished by the results recorded from the denervated facial and tongue muscles on stimulation of their autonomic nerve supply (figs. 10, 11, 12 and 13). These autonomic nerves do not end on the skeletal muscle fibers (cf. Hinsey, 1934, for references); they cannot therefore activate the muscle electrically. As Bremer and Rylant (1924) suggested, a humoral agent should be involved in the transmission. That this agent is acetylcholine is rendered very probable by the studies of Euler and Gaddum (1931) and Feldberg (1933b). The records illustrated in figures 10, 11, 12 and 13 constitute, therefore, instances of muscular propagated disturbances set up by acetylcholine liberated by nerve impulses, without any concomitant electrical activation. The latency of the responses to single nerve volleys in figure 13 is relatively long, about 60 msec., but this time is probably the time necessary for diffusion of acetylcholine from relatively distant sites to the motor plates. If an analogous mechanism is responsible for the transmission of the motor nerve impulse, as seems quite probable, the quanta of acetylcholine would be liberated at a much closer vicinity to the reacting structures, and a shorter delay would ensue. Indeed, the delay for the first spike in the responses to stimulation of the lingual may not be as long as that of the

last spikes of the responses of the facial muscles on stimulation of the VIIth nerve (Rosenblueth and Morison, 1937b).

The responses in figure 13 are repetitive and long-lasting, but it must be remembered that they were obtained after eserine, and that motor nerve volleys also elicit repetitive, long-lasting responses from their corresponding muscles after eserine (Brown, Dale and Feldberg, 1936).

Because of the technical difficulties of recording the mechanical responses of the tongue muscles with any accuracy, such records were not attempted. It is not possible, therefore, to deny that a contracture may have succeeded the contraction elicited by the single nerve volleys in figure 13, and that this contracture was responsible for the silent period which succeeds the initial spike outburst. This view is unlikely, however, for when acetylcholine elicits a contracture and silences the electrograms (fig. 5) the return of the electrical activity is gradual and slow, particularly after eserine (p. 788). In figure 13 the return of electrical activity after the brief silent period is sudden, and this activity is greater than that existing before application of the stimulus. It appears, therefore, more reasonable to attribute the silent period to a concentration of acetylcholine reaching at its peak the paralytic level of the motor plates of the muscles and causing a sudden stop of their activity (cf. fig. 7).

SUMMARY

The spontaneous electrical activity of cat's denervated skeletal muscles is temporarily increased after injections of eserine or prostigmin (fig. 1). This increase is simultaneous with a brief contraction (fig. 2).

Acetylcholine evokes contractions and contractures of the denervated muscles (figs. 3, 4 and 5). A delayed increase of electrical activity is sometimes recorded after acetylcholine (fig. 6). The spike potentials which attend the contractions cease abruptly with large doses (fig. 7). Acetylcholine may also render the muscles refractory to direct electrical stimulation (Gasser and Dale, 1926); this refractoriness is independent of the contractures (figs. 3 and 4, p. 788).

Tetani change the electrical excitability of the muscles, whether the tetani be elicited by direct electrical stimulation or by acetylcholine (fig. 9, p. 789). The responses to acetylcholine are increased after tetanic stimulation (fig. 8).

The contraction of the denervated tongue or face muscles on stimulation of the chorda tympani or cervical sympathetic (phenomena of Philipeaux-Vulpian and Rogowicz) is the result of typical conducted disturbances evoked by the autonomic nerve impulses (figs. 10, 11 and 12). Single shocks applied to the chorda tympani after eserine may cause the appearance of repetitive spike potentials (fig. 13).

These results support Dale and Feldberg's (1934) theory of chemical

mediation of the motor nerve impulses by acetylcholine (p. 795). Some of these results are not satisfactorily explained by the theory of electrical transmission (see p. 795).

REFERENCES

BOYD, T. E. This Journal **100:** 569, 1932.
BREMER, F. AND P. RYLANT. Compt. rend. Soc. Biol. **90:** 982, 1924.
BRISCOE, G. The Lancet **1:** 469, 1936.
BROWN, G. L. J. Physiol. **89:** 220, 1937a. Ibid. **89:** 438, 1937b.
BROWN, G. L., H. H. DALE AND W. FELDBERG. Ibid. **87:** 394, 1936.
COWAN, S. L. Ibid. **86:** 61P, 1936.
DALE, H. H. AND W. FELDBERG. Ibid. **81:** 39P, 1934.
DALE, H. H., W. FELDBERG AND M. VOGT. Ibid. **86:** 353, 1936.
EULER, N. S. AND F. H. GADDUM. Ibid. **73:** 54, 1931.
FELDBERG, W. Pflüger's Arch. **232:** 75, 1933a. Ibid. **232:** 88, 1933b.
FRANK, E., M. NOTHMANN AND H. HIRSCH-KAUFMANN. Ibid. **197:** 270, 1922.
GASSER, H. S. AND H. H. DALE. J. Pharmacol. Exper. Therap. **28:** 287, 1926.
GRUNDFEST, H. J. Physiol. **76:** 95, 1932.
HINSEY, J. C. Physiol. Rev. **14:** 514, 1934.
HOFMANN, F. B. Pflüger's Arch. **93:** 186, 1903.
LANGLEY, J. N. J. Physiol. **33:** 380, 1905.
LANGLEY, J. N. AND T. KATO. Ibid. **49:** 410, 1915.
LAPICQUE, L. L'excitabilité en fonction du temps. Paris, 1926.
PARKER, G. H. Proc. Nat. Acad. Sci. **20:** 306, 1934.
PHILIPEAUX, J. M. AND A. VULPIAN. Compt. rend. Acad. Sci. **56:** 1009, 1863.
ROGOWICZ, N. Pflüger's Arch. **36:** 1, 1885.
ROSENBLUETH, A., D. B. LINDSLEY AND R. S. MORISON. This Journal **115:** 53, 1936.
ROSENBLUETH, A. AND R. S. MORISON. Ibid. **119:** 236, 1937a. Ibid. **120:** 384, 1937b.
RUSHTON, W. A. H. J. Physiol. **77:** 337, 1933.
SCHÄFFER, H. AND H. LICHT. Arch. Exper. Path. Pharmakol. **115:** 180, 1926.
SCHIFF, J. M. Muskel- und Nervenphysiologie. Lahr, p. 189, 1858.
WATTS, C. F. J. Physiol. **59:** 143, 1924.

THE RELATION BETWEEN SUMMATION AND INHIBITION IN A SPINAL REFLEX

SAUL BERNSTEIN

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

Received for publication July 2, 1937

Recent investigations of the influence of a brief subliminal stimulus on peripheral nerve have shown that the excitability, as tested by a second shock, changes rapidly from an initial supernormality to a longer subnormal or depressed stage (Kato and others, 1929a and b; Erlanger and Blair, 1931a and b).

This phenomenon has formed the basis for an explanation of the Wedensky effect in peripheral tissues by Kato and his co-workers (1929a and b). Wedensky (1903) had found that a fatigued neuro-muscular junction, or a narcotized region of nerve, which conducts each of a series of impulses at low frequency, may block all but the first of such a series at a sufficiently high frequency. Kato and his collaborators demonstrated that the block is attributable to the fact that each impulse of the series after the first reaches the narcotized region during the period of depressed excitability set up by its predecessor. The importance attached to Wedensky inhibition by a number of authors (*e.g.*, Keith Lucas, 1917; Forbes, Davis, and Lambert, 1930), suggested a re-examination of the data of reflex inhibition in the light of these new developments.

Descriptions of the phenomena of central summation and inhibition by the Sherrington school and by Bremer were particularly suggestive. In the low spinal frog, Bremer (1930a and b) found that a centripetal volley of nerve impulses, alone incapable of evoking more than a minimal reflex response, markedly facilitates the response to a second volley, the effect varying with the interval between volleys. The facilitative effect reaches a sharp maximum at stimulus-intervals of 4 to 7 msec., and as the interval is increased, the abrupt decline in facilitation is usually succeeded by a second phase of facilitation showing a maximum at 30 to 40 msec. By a change in experimental procedure, Bremer was later (1931) able to demonstrate the inhibitory effect of a centripetal volley on subsequent reflex activity, with a maximum inhibitory effect at 10 to 12 msec. after the volley. Essentially similar relations were found in the spinal cat by Eccles and Sherrington (1930, 1931c).

These and a variety of related results have been described in terms of two conditions assumed to be produced at the junction between neurones, and named the "central excitatory state (c.e.s.)", and "central inhibitory state (c.i.s.)", respectively (Sherrington, 1925; Eccles and Sherrington, 1931c; Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932). The "central excitatory state", like Keith Lucas' "local excitatory state", is the condition of raised excitability produced by a subliminal stimulation. It is in this sense that the term will be used in this paper. (See Eccles 1935b for a broader usage.)

Out of all these facts the hypothesis seems to grow naturally that the condition of raised excitability produced by the subthreshold stimulation of one neurone by another is normally followed by a period of depressed excitability, just as is now known to be the case in peripheral nerve. Put in another way, it would mean that c.e.s. at any point on the neurone changes over into c.i.s., probably relatively prolonged, before the effect of the stimulation disappears. In terms of visible reflex phenomena, it would follow that if the impulses of a subliminal centripetal volley impinge relatively simultaneously upon a pool of motor neurones, whatever period of facilitation they provide for a subsequent volley should be followed by an inhibitory period.

In the researches of Bremer, and of Eccles and Sherrington, facilitation and inhibition were never studied in one and the same animal. As a check on the hypothesis, it was necessary to see whether in a given animal at a given time a single volley would produce a phase of inhibition following each of the two periods of facilitation. Exploratory experiments showed that the ipsilateral sciatic-triceps preparation used by Bremer would fulfill the requirements for examining the hypothesis, provided a day or more were allowed to intervene between the transection of the cord and the reflex experiments. This was necessary in order that excitability might recover to the point where a single strong shock would evoke a good reflex twitch. Thus both facilitation and inhibition produced by a volley could be tested by a single subsequent volley which would be facilitated, inhibited, or unaltered, depending upon the interval between volleys.

METHOD. In all of the experiments to be described, transection of the spinal cord of the frog (*Rana pipiens*) was performed between the third and fourth vertebrae. One to five days later, the animal's leg was dissected in preparation for the experiment, which was not begun until after the lapse of several hours, in order to permit the effects of the ether to wear off. Branches of the sciatic nerve to muscles other than the ipsilateral triceps femoris were cut. Circulation through the triceps was maintained intact. The leg was immobilized by pins driven through the bone at pelvic girdle and knee. Spontaneous movements of the anterior portion of the body were practically eliminated by means of a holder consisting essentially of a pair of U-shaped battery lugs clamped over the forelegs.

The sciatic nerve, cut at the knee, was enclosed in a small glass moist chamber of

the Sherrington type, and stimulated through a pair of Ag-AgCl-Ringer wire electrodes. The stimuli were brief condenser discharges delivered by a gas-filled triode stimulator (fig. 1). A counter-clockwise turn of the rotary switch, *S*, charges the stimulating condensers; a subsequent clockwise turn discharges each condenser through its respective tube. The first condenser is discharged simultaneously with the making of contact by the switch; the second, after a longer or shorter interval, depending upon the value of the resistance, *R*, in the delay circuit. Intervals of from about 1 to 300 msec. are thus obtainable, and longer intervals can be obtained by twice charging and discharging only one of the stimulating condensers. The shocks are free from chatter effects by virtue of the action of the tubes, which continue to pass current, once it has begun to flow, as long as the plate potential remains above a limiting value (about 15 volts), regardless of the grid potential. The time constants (RC) of the discharges range from 5 μsec. to 105 μsec., accommodation effects being therefore negligible. Even these time constants are longer than the actual "equivalent" time constants, because of the above-mentioned action of the tubes in cutting off the "tails" of the discharges.

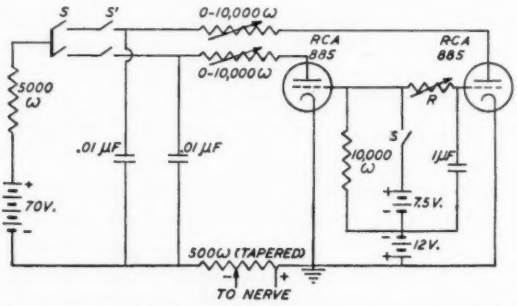


Fig. 1. Circuit diagram of stimulator. *S*, *S*, parts of same double-throw, center-off rotary switch. *S'*, separate switches for throwing either shock into or out of action. *R*, decade resistor, 1000–400,000ω. *RCA* 885, gas-filled triodes. Remainder of description in text.

Contractions of the muscle were recorded isometrically by means of a strip of steel watch-spring, clamped at one end, and provided with a straw and writing-point at the other. Two springs were used: a relatively stronger one, for recording muscle twitches evoked by stimulation of the motor nerve; and a weaker one, for reflex responses. The natural frequencies were respectively 110 and 80 d.v./sec.; shortening of the muscle permitted by the springs was 0.05 mm./100 gm. and 0.26 mm./100 gm., respectively. Both springs were thinly coated with solder to increase damping.

The muscle responses were recorded on smoked kymograph paper moving at the rate of 110 mm./sec. in most of the experiments. The area of the reflex twitch curve was taken to be more accurate than the maximal height of contraction as an index of the magnitude of reflex nervous response (total number of impulses set up reflexly in the motor nerve). Measurements of height (tension) alone fail to take into account the dispersion of impulses typically occurring in the reflex twitch and seen most strikingly as after-discharge. For example, in the case of a typical reflex response such as that shown in figure 2, *B*, inhibition of the impulses set up earlier in the discharge might markedly reduce the maximal height of the curve, while no such effect would occur if the later impulses were thus eliminated.

To aid in the measurement of the areas, the records were rendered translucent by being passed through a bath of lubricating oil, and enlarged for planimeter measurement by means of a lantern-slide projector. Each recorded value for the area of a curve is the mean of five planimeter measurements. Before the technique was developed of measuring isometric twitch areas, a number of experiments were limited to the recording of isotonic contraction heights on a stationary drum.

Shocks of the desired intensities were delivered in pairs, with varied intervals between the members of a pair. The interval between pairs, however, was maintained constant throughout a given experiment, at a duration (½–3 minutes) which would insure complete recovery before each new stimulation. As a check on unavoidable variations of excitability, single shocks of constant intensity were thrown in at fairly regular intervals throughout the experiment, in order that the records

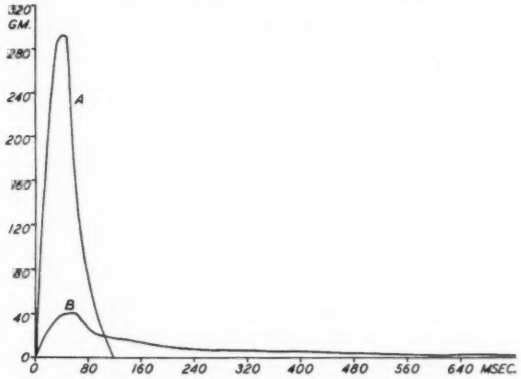


Fig. 2. *A*, isometric twitch of triceps, in response to a single maximal stimulation of the sciatic as motor nerve (frog 61, record 32). *B*, isometric reflex twitch of triceps, in response to a single submaximal shock to the sciatic as afferent nerve (frog 62A, record 3). The remainder of the tail of the curve is not shown. Total duration of the curve, about 1.25 sec.

Ordinates, tension developed, in grams; abscissae, duration of response, in milliseconds. The curves were obtained with different spring levers, but have been redrawn to the same scale.

so obtained might be used to plot a curve of variation of responsiveness during the experiment. The experimental curve, consisting of the area of the response plotted against the interval between shocks, was then corrected, if necessary, in terms of the variation curve.

The experiments were all performed at room temperature, which was usually above 20°C. Records taken at 10-minute intervals throughout each experiment showed that the temperature almost never varied so much as two degrees, and usually not more than a few tenths of a degree, during a given experiment. A single experiment lasted from ½ to 3 hours, and the frog was usually in condition for a second and a third experiment.

RESULTS. Four types of experiments were carried out on the sciatic-triceps reflex, differing with respect to the intensities of the first and

second shocks of a pair. In addition, controls were performed on the gastrocnemius, and on the triceps, stimulated through their motor nerves. In any given experiment, the intensities of the two shocks were maintained constant throughout, the interval between them being the only variable.

Type I. Each stimulus of the pair inadequate to produce a reflex response. In all seventeen of these experiments, isotonic reflex responses were recorded on a stationary drum. Unlike the other experiments to be described below, these were carried out on frogs whose cords had been transected only an hour or two before the experiment. A typical height-interval curve (height of contraction plotted against the interval between the shocks of a pair) is shown in figure 3, *I*. It presents a sharp summation peak at 8 to 10 msec., and a second, lower peak at 60 to 70 msec. The rôle of this type of experiment in the general argument to be developed is not essentially weakened by the fact that heights rather than areas of responses were used. The agreement in general form of this type of curve with the area-interval curves derived from the three other types of experiment, is sufficiently clear from figure 3. The first peak in about half the cases occurred at 3 to 5 msec., and the second maximum occasionally fell at an interval as long as 125 msec., though usually occurring regularly at 50 to 75 msec. In rare cases, particularly where the frog's general level of excitability was low, only the first peak was obtained. While these results simply confirm Bremer's observations, they are of importance in the analysis of the other experiments.

Type II. Each shock of the pair maximal in the sense of evoking the largest reflex response capable of being evoked by a single shock. Figure 3, *II*, shows the characteristic area-interval curve (area of second response plotted against the interval between stimuli of a pair) yielded by the nine experiments of this type. The "area of the second response" is the total area of the response to both shocks of a pair, less the area of the response to the first shock alone. The two summation peaks correspond roughly with those of figure 3, *I*, but in addition, two depression periods make their appearance: a brief, fairly deep one following the sharp summation peak, and a prolonged, gradual one following the second, more rounded peak. During these "depressions" the second shock of a pair evokes a response of smaller area than it does when unconditioned by the first shock.

Only two long intervals were used in the experiment (frog 58) from which figure 3, *II*, is derived. At 4.8 sec., the second response was subnormal; at 46 sec., it was normal (*i.e.*, the area was equal to that of the unconditioned second response, represented by the horizontal line of dashes in fig. 3). More complete data on the second period of depression, derived from a similar experiment, indicate that recovery may not be complete for 17 sec. or more. While the data at hand on the precise

course of the second depression are rather meager, they admit of no doubt as to its reality.

Type III. First stimulus barely adequate to produce a reflex response, second evoking a submaximal response. Only two curves of this type have been obtained, and those are incomplete beyond 250 msec. As indicated

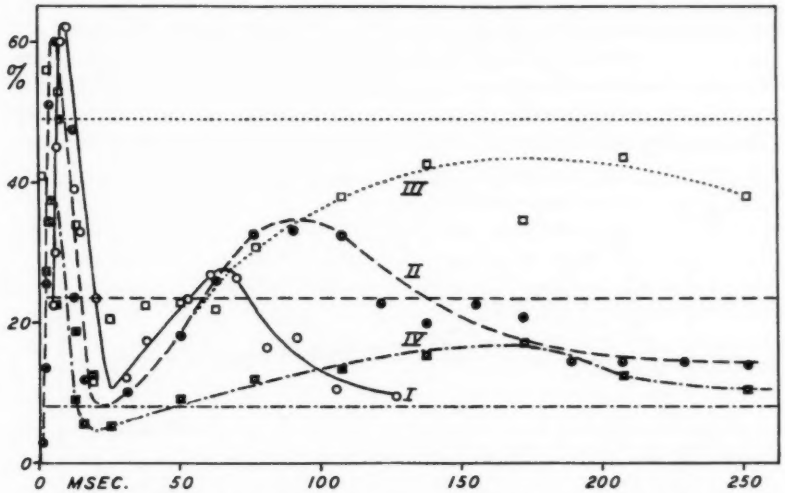


Fig. 3. Typical sciatic-triceps reflex response-interval curves yielded by the four types of experiments described in the text.

Type I (*blank circles*), each shock reflexly subliminal. Ordinates, isotonic contraction heights (10 mm. arbitrarily = 30 per cent on ordinate scale); abscissae, interval between shocks of a pair, in milliseconds. Frog 20B. Temp., 24.8–25.0°C.

For the other curves: ordinates, area of isometric contraction curve due to the second shock of a pair, as per cent of area of maximal motor twitch; abscissae, interval between shocks of a pair, in milliseconds.

Type II (*full circles*), each shock maximal. Frog 58. Temp., 19.1–20.5°C.

Type III (*blank squares*), first shock just reflexly supraliminal, second submaximal. The first portion of the curve (up to 50 msec.) practically coincides with that of type II. Frog 62B. Temp., 23.0–23.6°C.

Type IV (*full squares*), each shock just reflexly supraliminal. Up to 5 msec., the curve practically coincides with that of type II. Frog 63. Temp., 22.0–22.3°C.

The area of the unconditioned response to the second (testing) shock alone in each experiment is represented as a horizontal line of the same kind as is used for the corresponding curve.

in figure 3, *III*, they show a first summation peak and first depression capable of being represented by the type II curve. By 250 msec., there are indications of the dip into the second, prolonged depression. Although the curve, as far as it goes, bears close similarities to the preceding type, it is to be noted that actual summation is obtained only with shock inter-

vals of 3 to 10 msec. The second elevation in the curve does not reach normal at all (that is, the size of the conditioned second response does not attain that of the unconditioned response, which is represented by the horizontal dotted line).

Type IV. Each stimulus of the pair barely adequate to evoke a reflex response. Of this type of area-interval curve, I have obtained only one good specimen, shown in figure 3, *IV*. It agrees fairly well with the results of a number of experiments performed early in the work, using the same relative strength of stimulation, but involving isotonic heights instead of isometric areas. Both summation peaks are well marked, but the first depression is very small. The rising phase of the first summation

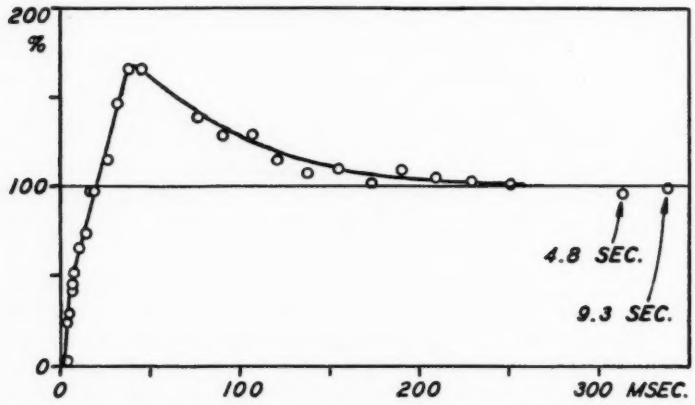


Fig. 4. Recovery curve of triceps femoris muscle, stimulated through the sciatic as motor nerve. Each shock of pair supramaximal. Ordinates, area of isometric contraction curve due to the second shock of a pair, as per cent of area of maximal single twitch; abscissae, interval between shocks of a pair, in milliseconds. Frog 61. Temp., 23.2-23.4°C.

peak is represented in the figure by the corresponding portion of the type II curve. The second depression (not shown in the figure) is relatively profound, lasting for some 20 sec., with its low point at 10 sec.

Nerve-muscle records, as controls; each volley maximal. These experiments were performed on preparations in all respects similar to those described above, except that the sciatic was used as a motor, rather than as an afferent nerve. Essentially similar results were obtained with the gastrocnemius and the triceps. The area-interval curve for the triceps, shown in figure 4, agrees well with the height-interval recovery curve reported by Hartree and Hill (1921). The peak of supernormality in figure 4 comes at 40 to 50 msec., normal excitability being regained at about 250 msec. No significant descent of the summation curve below

normal excitability after this point was ever observed in twenty experiments of this kind.

Analysis of the data. If the hypothesis be correct that the central excitatory state set up at a neurone by impulses which fail to excite it is regularly followed by a central inhibitory state, every period of reflex facilitation in a response-interval curve should be followed by a period of depressed responsiveness, provided the experimental conditions are such that both facilitation and inhibition can be manifested.

In those of the experiments described above in which a depressed period is capable of being manifested (that is, in all except type I), the data, so far as they go, seem to meet this basic requirement of the hypothesis. Analysis is required, however, to determine to what extent, if any, the two summation peaks of the curves are really manifestations of c.e.s.; and the two depression periods, of c.i.s. The assumption seems reasonable that the familial resemblances of all four curves of figure 3 are based upon a fundamental similarity of the underlying processes. It is therefore proposed to consider in turn the important features shown by the family of curves as a whole, rather than to analyze each curve separately.

The first question to consider is whether the *first peak*, which regularly appears in all four types of experiment, actually represents the existence of c.e.s. set up by the first shock. Curves of type I, in which each shock alone fails to evoke any reflex response, prove conclusively that summation, either at the site of stimulation or centrally, is responsible for the first peak. The properties of the muscle are not involved, since the first shock of each pair produces no effect on the muscle. Results closely similar to these have been obtained in spinal cats by Eccles and Sherrington (1931c), both when the pair of stimuli were delivered through the same electrodes, and when they were sent in through two separate afferent nerves. In the latter case, the possibility is excluded that the summation occurs at the point of stimulation.

The curves of Eccles and Sherrington obtained by stimulation through a single pair of electrodes differ essentially from those obtained by the stimulation of two separate nerves, in only one respect: they show a sharp rise to a peak, whereas the latter apparently begin at maximum with simultaneity of the pair of shocks. Now the data of Gasser and Erlanger (1930, p. 269) show an absolutely refractory period of less than 1 msec., and a relatively refractory period ranging from 3.5 to 8 msec., in the sciatic nerve of the "green frog" at 20° to 24°C. Obtained under nearly identical conditions, the curves of figure 3 show a rising phase of the first peak which starts at less than 1 msec., and attains a maximum at 3 to 8 msec. Thus there can be little doubt that the phenomenon is attributable to the absolute and relative refractoriness of the peripheral nerve at the locus of stimulation. (Eccles and Sherrington, 1931c; Bremer, 1930a;

Eccles, 1935b.) But if the first sharp rise of the curve of type I is due to refractoriness at the point of stimulation, this must mean that a volley of impulses has been set up in the nerve by the first shock, which was inadequate to produce a reflex response. In addition, the experiments exemplified by figure 3, *II*, in which each stimulus alone evoked the largest reflex response obtainable with a single shock, demonstrate that the first peak is due to central facilitation.

It is important to note that supernormality of excitability of motor neurones following their discharge by the first volley of a pair may be ruled out as an essential factor in producing either the first or the second peak. When the first shock of a pair calls forth no reflex response, motor neurone supernormality cannot be involved, since no motor neurones have been excited. Again, when both volleys are maximal, the peaks rise above the value for the unconditioned second response. That must mean that new muscle fibers, hence new motor neurones, have been recruited in addition to part or all of those initially discharged by the first volley. For if the all or nothing principle holds, the response of a muscle fiber will be no greater when excited through a supernormal neurone than through a normal or subnormal one.

We may therefore conclude that the first peak is a manifestation of c.e.s., and that its rising phase is largely determined by the refractoriness of peripheral nerve at the locus of stimulation.

As to the locus of the c.e.s., three possibilities exist: either it is set up at the motor neurones themselves, or at internuncial neurones, or both. Histological preparations by Cajal have proven the existence of direct synaptic connections between afferent neurones entering the cord by way of the dorsal roots, and motor neurones, in the frog (Kappers, Huber, and Crosby, 1936, v.1, p. 186). The experiments of Eccles (1931) on the flexor reflex of the cat (in which results have been obtained closely similar to those yielded by the sciatic-triceps reflex of the frog), have led him to the conclusion that "in any motoneurone an antidromic impulse removes the c.e.s. which it finds preformed"; hence, the c.e.s. must be built up at the motor neurone. While the occurrence of c.e.s. at internuncial neurones is not excluded by the evidence, the data of Eccles (1931) suggest that such c.e.s. plays a subsidiary rôle, if any. It may be assumed, then, with Bremer (1933), as the simplest hypothesis, and one supported by indirect evidence, that *the c.e.s. responsible for the first peak is built up at the motor neurones for the most part if not entirely.*

The same set of arguments that has been used to establish the conclusion that the first peak is a manifestation of c.e.s., applies with full force to the analysis of the *second peak.* But a new possibility now appears. The peak of the muscle recovery curve as obtained by maximal stimulation of the motor nerve (fig. 4), occurs at 40 to 50 msec. While this falls far

short of coinciding in time with the second peaks of the area-interval curves, it is nevertheless sufficiently close to suggest a possible relationship. That it is not a major factor in producing the second elevation, however, is clear from the fact that it is present in curves of types I and IV (fig. 3), where the first shock of a pair produces no twitch at all, or only a very small one. If no muscle fibers are set into activity by the first volley, muscle supernormality cannot affect the response to the second. Hence, while muscle recovery may and probably does influence the form of curves such as that of figure 3, *II*, where the first shock of a pair evokes a considerable reflex response, it cannot be an essential factor in producing the peak. Certain other proposals to account for the peak have apparently been adequately dealt with by Bremer (1930b). Presumably, then, *the second peak is, like the first, essentially a manifestation of c.e.s.*

The second elevation in the area-interval curves lasts so long (150–250 msec.) after the initial volley of a pair as to make it extremely improbable that it is merely the prolongation of the effect of the same group of impulses (arriving at the motor neurones) that were responsible for the first peak. Evidence from experiments by Eccles (1931), using antidromic volleys, shows that "there is a temporal dispersion of the excitatory impulses incident on the motoneurones as the result of a single centripetal volley". Eccles and Sherrington (1931a) state further that "a period of quiescence follows an antidromic volley set up during the after-discharge of a reflex evoked either by a single centripetal volley or by a repetitive series of centripetal volleys". The after-discharge of a typical sciatic-triceps reflex response to a single shock in the frog, as represented in figure 2, *B*, lasts well over a second, and sometimes persists for several seconds. *Since this after-discharge can be accounted for in no other way than by the continual arrival of delayed impulses at the motor neurones, the inference is obvious that the second peak of our area-interval curves owes its existence to c.e.s. developed in this manner.* That this "delayed c.e.s.", as Eccles and Sherrington call it, is built up at motor, rather than at internuncial, neurones, is demonstrated by the above-quoted effect upon it of an antidromic volley sent up the motor nerve. The apparently reasonable suggestion is made by Bremer that the delay paths include internuncial neurones, in accordance with the histological facts.

To suppose, however, that the centripetal impulses arrive at the motor neurones in two groups as distinct as are the first and second peaks of the curves of figure 3, would be not to take into account the possibility of concomitant inhibition, to which we now turn.

The *first depression* of the area-interval curves is a manifestation either of refractoriness (irresponsiveness consequent upon previous activity), or of inhibition, if we accept Hughes and Gasser's (1934) working definition of inhibition as "a response-deficit not directly attributable to refrac-

toriness". That refractoriness of the motor neurones discharged by the first volley of a pair must play a part proportionate to the initial response in determining the response to a second volley, is not to be denied. Yet in type III, figure 3, a marked depression is produced in an otherwise relatively strong response, by an antecedent volley which can cause almost no refractoriness because it excites only a very small number of motor neurones. Again, in the experiments of Bremer (1931), depression of the sciatic-triceps reflex is produced by a centripetal volley which alone evokes no response, hence involves no refractoriness of motor neurones. The change of responsiveness in this case follows a course very similar to that of our "first depression". The assumption seems reasonable, therefore, on the basis of the strong resemblances of our four types of experimental curves, *that the first depression is largely, if not entirely, a true inhibitory manifestation; i.e., that it represents the development of c.i.s.*

As to whether the c.i.s. develops at the motor neurones, there is no direct proof at present. However, if two-neurone arcs are involved in the frog's sciatic-triceps reflex, as they seem to be in the cat's flexor reflex, there is no other locus at which c.i.s. could develop, as far as centripetal impulses using such paths are concerned. The reasons for regarding two-neurone arcs as likely in the frog's sciatic-triceps reflex have been presented above. The schema proposed by Hughes and Gasser (1934) to account for inhibition in terms of refractoriness of internuncial neurones, seems to require more than two neurones in such arcs. *On the whole, the evidence, while inconclusive, suggests that the c.i.s. responsible for the first depression develops at the motor neurones.* Concomitant refractoriness of internuncial neurones is not excluded, however.

The fact that a well marked phase of inhibition occurs in the period between the first and second peaks of the area-interval curves, indicates that the falling phase of the first peak, and the rising phase of the second, must be largely determined by the degree of development of c.i.s. It would therefore probably be a mistake to regard the peaks as manifestations of two widely separated groups of impulses, one group going directly from afferent fiber to motor neurone, the other by way of an internuncial neurone. It would seem more likely that algebraic summation of inhibitory and excitatory effects occurs at any given interval to determine the size of the response.

With regard to the *second, long depression,* the question likewise arises as to whether it is attributable in the main to refractoriness or to c.i.s. The evidence on this point is not as yet available.

So far, then, if the data and the analysis are substantially correct, the following conclusions may be drawn with a considerable degree of probability. In the sciatic-triceps reflex of the low spinal frog, a centripetal volley of whatever size sets up at the motor neurones: *1,* a brief period

during which c.e.s. tends to preponderate strongly, followed by *2*, a somewhat longer period during which c.i.s. tends to preponderate; *3*, a still longer period during which c.e.s. once more overbalances c.i.s., though attaining a lesser value than in the first period. This stage gives place to a prolonged depression phase, which may or may not involve the existence of c.i.s. at the motor neurones. So far as the data go, and in the light of related work previously done, they fulfill the requirements of the hypothesis of the changing over of c.e.s. to c.i.s. In no way do the data appear to contradict this hypothesis.

Discussion. Separate inhibitory and excitatory fibers have been assumed to occur in afferent nerves by various authors (Eccles and Sherrington, 1931c; Hughes and Gasser, 1934), on the basis of more or less indirect evidence. It was not, however, until Kato and his co-workers (1934) attacked the problem through preparations of single afferent fibers, that indisputable proof was given of the existence of these distinct categories of fibers.

At first sight, this demonstration seems to contradict the possibility of the changing over of c.e.s. to c.i.s., since if the latter is true, a single fiber may produce both excitatory and inhibitory effects. There is no basic contradiction involved, however, provided the afferent fiber be carefully distinguished from its central endings on other neurones. The existence of *peripheral fibers* which are specifically inhibitory or excitatory *for given reflexes* is perfectly compatible with the c.e.s.-c.i.s. change; the existence of specifically inhibitory or excitatory *endings* on neurones is not. The latter possibility has never been shown to be true for the central nervous system of Vertebrates.

The fact of inhibitory and excitatory fibers, and the hypothesis of c.e.s.-c.i.s. change, may be brought into agreement by the assumption that an afferent fiber which is excitatory for a given motor neurone leads to it through a different type of path from that connecting an inhibitory fiber with the same motor neurone. The essential feature is that impulses arriving at the motor neurone from the inhibitory fiber must arrive at a sufficiently low frequency, relative to those arriving via the excitatory fiber. If each impulse arriving at an ending on the motor neurone is alone inadequate to discharge the neurone—and all the evidence indicates that this is the case (cf. Eccles, 1935a and b)—then those arriving at a sufficiently high frequency will arrive during the c.e.s. set up by their predecessors, and will sum to produce excitation. If impulses arrive at so low a frequency that each falls in the c.i.s. set up by its predecessor, the c.i.s. will be maintained or increased, thus tending to prevent excitation even by a normally excitatory series of impulses. This is precisely the explanation furnished by Kato and his co-workers for Wedensky inhibition.

A corollary of this assumption is that the same fiber that is excitatory

for one motor neurone may be inhibitory for another, by virtue of a different set of central connections. Just this relationship has been found in the spinal cat by Kato, Kaku, and Tasaki (1935). A single afferent fiber has been shown to be capable of exciting ipselateral flexor muscle fibers, while at the same time inhibiting ipselateral extensor fibers.

It is worthy of mention that Eccles, whose earlier experiments (1935a and b) on the cervical sympathetic ganglion of the cat had led him to postulate the existence of distinct excitatory and inhibitory preganglionic fibers, has since (1936) abandoned this view in the light of work with antidromic impulses. He favors the conclusion that the same fiber may produce inhibitory and excitatory effects. It is important to observe that the synaptic relations between the preganglionic fibers and the ganglion cells are all fairly simple and similar; no internuncial neurones are present.

Nothing has been said so far of the nature of the change from c.e.s. to c.i.s. so frequently mentioned. Studies of similar changes from excitatory to inhibitory state in peripheral nerve suggest that these phenomena are closely bound up with those of electrotonus; and particularly with what has been called "accommodation". Accommodation is the gradual rise of threshold that is produced in peripheral nerve, for example, in response to a prolonged subliminal cathodal stimulation. There seems to be no reason to differentiate between the "accommodation" produced by a prolonged stimulation, and the "depression" (Erlanger and Blair, 1931a and b) produced by a brief one of the same kind. Sherrington's remark (1929) that "inhibition may be the stabilising of a surface film" may be recalled.

SUMMARY

From the fact that a brief, subliminal cathodal shock sets up a phase of raised excitability followed by one of depressed excitability at the site of stimulation in peripheral nerve (Kato and others; Erlanger and Blair); and from the data yielded by experiments on spinal cord reflexes (Bremer; Eccles and Sherrington), the hypothesis is formed that the central excitatory state (c.e.s.) set up at a motor neurone by a subliminal volley of impulses, is normally succeeded by a central inhibitory state (c.i.s.) at the same locus.

Single condenser shocks delivered to the sciatic nerve evoke a strong reflex response of the triceps femoris muscle in frogs which have been given time to recover from the effects of transection of the spinal cord just anterior to the lumbar region. In such frogs, the first shock of a pair may facilitate or inhibit to varying degrees the response to the second shock, depending upon the time intervals between shocks, their rela-

tive and absolute intensities being maintained constant in any given experiment.

When both shocks are maximal in intensity, the second response tends to be strongly facilitated by the first shock at intervals of about 2 to 15 msec., with a maximal effect at about 5 msec. This phase changes over into a period of depression showing a maximal effect at about 20 to 25 msec. A second facilitation period follows, with a lower maximum at roughly 100 msec., giving place finally to a second depression period lasting for several seconds at least. The results obtained at other absolute and relative intensities of stimulation show similar features.

Detailed analysis of the records leads to the conclusion that the hypothesis of c.e.s.-c.i.s. change is supported by the evidence, though not proven to the exclusion of all other possibilities.

The relation of the hypothesis to the existence of distinct excitatory and inhibitory afferent nerve fibers is discussed, and the conclusion is reached that there is no inherent contradiction between the facts and the hypothesis, provided the fibers in the nerve trunk be not confused with their central endings.

I wish to express my thanks to Prof. Kenneth S. Cole and to Prof. H. B. Williams for many valuable suggestions and much encouragement during the course of the work.

REFERENCES

BREMER, F. C. R. Soc. Biol. **103:** 509, 1930a.
C. R. Soc. Biol. **104:** 810, 1930b.
C. R. Soc. Biol. **106:** 465, 1931.
Ann. de physiol. et de physico-chimie biol. **9:** no. 4, 1933.
CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL, AND C. S. SHERRINGTON. Reflex activity of the spinal cord. Oxford, 1932.
ECCLES, J. C. Proc. Roy. Soc. B **107:** 557, 1931.
J. Physiol. **85:** 207, 1935a.
J. Physiol. **85:** 464, 1935b.
J. Physiol. **88:** 1, 1936.
ECCLES, J. C. AND C. S. SHERRINGTON. J. Physiol. **69:** 1, 1930.
Proc. Roy. Soc. B **107:** 535, 1931a.
Proc. Roy. Soc. B **107:** 586, 1931b.
Proc. Roy. Soc. B **107:** 597, 1931c.
Proc. Roy. Soc. B **109:** 91, 1931d.
ERLANGER, J. AND E. A. BLAIR. This Journal **99:** 108, 1931a.
This Journal **99:** 129, 1931b.
FORBES, A., H. DAVIS AND E. LAMBERT. This Journal **95:** 142, 1930.
GASSER, H. S. AND J. ERLANGER. This Journal **94:** 247, 1930.
HARTREE, W. AND A. V. HILL. J. Physiol. **55:** 389, 1921.
HUGHES, J. AND H. S. GASSER. This Journal **108:** 307, 1934.

KAPPERS, C. U. A., G. C. HUBER AND E. C. CROSBY. The comparative anatomy of the nervous system of vertebrates, including man. New York, 1936.

KATO, G. The microphysiology of nerve. Tokyo, 1934.

KATO, G., T. HAYASHI, T. OTA, M. NAKAYAMA, H. TAMURA, M. TAKEUCHI, K. KANAI AND S. MATSUYAMA. This Journal **89:** 471, 1929a.
This Journal **89:** 692, 1929b.

KATO, G., Z. KAKU AND I. TASAKI. Summary of communications, XVth Internat. Physiol. Cong., p. 194. Leningrad, 1935.

LUCAS, K. The conduction of the nervous impulse. London, 1917.

SHERRINGTON, C. S. Proc. Roy. Soc. B **97:** 519, 1925.
Proc. Roy. Soc. B **105:** 332, 1929.

WEDENSKY, N. Pflüger's Arch. **100:** 1, 1903.

MEDICAL LIBRARY DEC 7 1937

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

The Relation of the Hypophysis and Adrenal Cortex to the Removal of Excess Glucose from the Blood of Rats. *Leo T. Samuels, Hermann F. Schott and Howard A. Ball* 649

The Effect of Atropine on Gastric Secretion and Its Relation to the Gastrin Theory. *John S. Gray* 657

The Origin of the R and T Potentials from the Mammalian Heart. *J. A. E. Eyster, M. R. Krasno, C. A. Maaske and M. J. Ulevich* 663

The Electrical Potentials of the Cochlea and Auditory Nerve in Relation to Hearing. *John Guttman and S. E. Barrera* 666

The Relation of Amniotin to the Basal Metabolism of the Thyroidectomized Rat. *T. C. Sherwood, T. M. Wilson and H. Boneta* 671

Loss of Potassium in Voluntary Contraction. *Wallace O. Fenn* 675

The Nitrogen-Sparing Action of Glucose in Normal, Phlorhizinized and Depancreatized Dogs. *I. Arthur Mirsky, Joseph D. Heiman and S. Swadesh* 681

Factors Which Influence Dark Adaptation. *Charles A. Elsberg and H. Spotnitz* 689

Serum and Urine Phosphatase Activity in the Cat after Ligation of the Common Bile Duct. *Charles A. Flood, Ethel Benedict Gutman and Alexander B. Gutman* 696

The Galvanic Nystagmus Reaction in the Monkey. *Page Northington and S. E. Barrera* ... 703

Effects of Mecholyl on Gastric Secretion. *John S. Gray and A. C. Ivy* 705

Rapid Decompression Following Inhalation of Helium-Oxygen Mixtures Under Pressure. *Edgar End* 712

Electrolyte and Water Changes in Muscle During Atrophy. *H. M. Hines and G. C. Knowlton* 719

Electrical Studies of Ovulation in the Rabbit. *J. Reboul, H. Davis and H. B. Friedgood* ... 724

Absorption of Insulin from Thiry-Vella Loops of the Intestine in Normal and Depancreatized Dogs. *John R. Murlin, Ruth Latta Tomboulian and H. B. Pierce* 733

The Effect of Hexylresorcinol upon the Absorption of Insulin from the Gastro-Intestinal Tract of Dogs. *R. G. Daggs, W. R. Murlin and J. R. Murlin* 744

Reflex and Humoral Gastro-Intestinal Inhibition in Unanesthetized Dogs During Rectal Stimulation. *Wm. B. Youmans and Walter J. Meek* 750

Acetylcholine Contracture of Denervated Muscle. *G. C. Knowlton and H. M. Hines* 757

A Relationship Between the Blood Sugar Level and the Rate of Sugar Utilization, Affecting the Theories of Diabetes. *Samuel Soskin and R. Levine* 761

The Functions of the Intra-Aural Muscles. *Harold C. Wiggers* 771

A Study of Denervated Mammalian Skeletal Muscle. *A. Rosenblueth and J. V. Luco* 781

The Relation Between Summation and Inhibition in a Spinal Reflex. *Saul Bernstein* 798

Index 813

VOL. 120—NO. 4

Issued December 1, 1937

BALTIMORE, U. S. A.

1937

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

THE AMERICAN JOURNAL OF PHYSIOLOGY

Editorial Policy. The Council has approved the following policy of management:

Manuscripts should be sent to the Managing Editor who will see that each paper is read by two or more members of the Editorial Board. Authors will then be advised as to the suitability of the paper or the desirability of any revision. The Editorial Board will be governed by certain general principles:

1. The suitability of papers will not be judged by arbitrary standards of length but on their content of significant new research results in physiology, presented with the greatest brevity which is compatible with scientific accuracy and clarity.

2. Preference will be given to papers from American Laboratories in the field of vertebrate physiology and to those which contribute to problems related to this field.

3. Subdivision of material coming out of a general research into two or more papers will be discouraged.

4. Papers restricted to the description of new apparatus or methods or which appear to be of the nature of progress reports, the publication of which might properly be withheld until the research has progressed to the completion of at least a significant phase of the problem, will not be accepted.

5. Papers giving confirmatory or negative results will be considered only if presented in the briefest possible space.

6. Since manuscripts will not be insured against loss or injury when being given editorial consideration, contributors will be expected to retain duplicate copies, either originals or photographs, of all material (manuscripts, illustrative and tabular matter) submitted for publication.

The following practical aspects are important in the preparation of papers:

a. Duplication of data in tables, charts and protocols is not believed to be generally necessary. Too extensive use of such material is likewise to be deprecated.

b. Tables and illustrative material should be prepared with the size of the Journal page ($4\frac{1}{2}$ x $7\frac{1}{2}$ inches) in mind, specifically with the idea of conserving vertical space.

c. It is advantageous, when feasible, to group illustrations. This should be done with as little waste space between the several units as is possible and also with the idea of conserving vertical space.

d. Since duplication of charts, graphic tracings, etc., is required in paragraph six above, this is best done by photographs of the size desired in reproduction and printed on glossy paper. Either the originals or photographs may be submitted. If the originals are larger than $8\frac{1}{2}$ x 11 inches they must in all cases be accompanied by photographic reproductions. When such photographs are adequate for good reproduction the originals need not be supplied.

e. Plotted curves and their guide-lines should be drawn in India ink on blue-lined coördinate paper.

f. All illustrative material must be submitted in such form as to admit of photographic reproduction without retouching, redrawing or the setting of marginal type.

g. References to cited papers should conform to the practice of the Quarterly Cumulative Index Medicus and should be in this order: Author's name, journal, volume (in Arabic), initial page, year.

EDITORIAL BOARD

W. M. Boothby — R. G. Hoskins
J. G. Dusser de Barenne — A. C. Ivy
Joseph Erlanger — A. N. Richards
W. E. Garrey — C. J. Wiggers

D. R. Hooker, *Managing Editor*
Laura E. Campen, *Secretary of Publications*
19 West Chase Street, Baltimore, Md.

The American Journal of Physiology is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about seven hundred and twenty pages, are published yearly. The subscription price per volume in the United States and Canada is $7.50; in other countries, $8.00.

PHYSIOLOGICAL REVIEWS

Contents of Volume 14, 1934

E. K. MARSHALL: The Aglomerular Kidney
L. B. FLEXNER: The Chemistry and Nature of the Cerebrospinal Fluid
H. JENSEN AND E. A. EVANS, JR.: The Chemistry of Insulin
R. G. SINCLAIR: Physiology of the Phospholipids
S. W. CLAUSEN: Diet and Infection
W. S. DUKE-ELDER: Application of Physiological Chemistry in the Study of Intraocular Pressure
J. M. PETERSON: Metabolism of the Eviscerate Preparation
E. M. LANDIS: Capillary Pressure and Capillary Permeability
J. C. HINSEY: The Innervation of Skeletal Muscle
G. A. ALLES: The Physiological Significance of Choline Derivatives
R. C. GARRY: The Movements of the Large Intestine
A. C. IVY: Physiology of the Gall Bladder
S. MUDD, M. MCCUTCHEON AND B. LUCKE: Phagocytosis

Contents of Volume 15, 1935

O. R. MCCOY: Physiology of Animal Parasites
C. L. A. SCHMIDT AND D. M. GREENBERG: Transport of Alkaline Earth Elements in the Animal Body
C. M. WILHELMJ: Specific Dynamic Action
W. F. VON OETTINGEN: Manganese, Its Distribution, Pharmacology and Health Hazards
W. H. HOWELL: Present Status of the Problem of Blood Coagulation
W. E. GARREY AND W. R. BRYAN: Variations in White Blood Cell Counts
C. A. ELVEHJEM: Iron and Copper Metabolism
C. E. BILLS: Physiology of Sterols and the Influence of Light on Sterols
W. M. HOSKINS AND R. CRAIG: Recent Developments in Insect Physiology
M. N. RICHTER AND E. C. MCDOWELL: Experiments with Mammalian Leukemia
P. WEISS: Organizers in Embryological Development
F. J. W. ROUGHTON: Recent Work on CO_2 Transport
R. J. S. MCDOWALL: The Nervous Control of the Blood Vessels

Contents of Volume 16, 1936

J. BARCROFT: Fetal Circulation and Respiration
W. J. V. OSTERHOUT: Electrical Studies on Large Plant Cells
W. O. FENN: Electrolytes in Muscle
A. N. DRURY: Physiological Activity of Nucleic Acid Derivatives
R. HÖBER: Membrane Permeability to Solutes in Its Relations to Physiology
L. B. AREY: Mechanism of Wound Healing
H. DAVIS AND A. FORBES: Chronaxie
A. P. KRUEGER: The Nature of Bacteriophage and Its Mode of Action
C. G. KING: Ascorbutic Acid and Vitamin C
H. J. DEUEL: Metabolism of Fructose and Galactose
W. O. NELSON: Lactation
D. B. DILL: Economy of Muscular Activity
R. S. ALCOCK: The Synthesis of Proteins in Vivo
JOHN H. FERGUSON. The Blood Calcium and the Calcium Factor in Blood Coagulation
H. G. WOLFF: The Cerebral Circulation
E. W. H. CRUICKSHANK: Metabolism of Cardiac Muscle
H. R. ING: Curariform Action of Onium Salts
L. EARLE ARNOW: Effects Produced by the Irradiation of Proteins and Amino Acids.
G. BOURNE: The Rôle of Vitamin C in the Organism as Suggested by Its Cytology
E. PONDER: The Kinetics of Hemolysis

PHYSIOLOGICAL REVIEWS

Contents of Volume 17, 1937

M. C. Bourne: Metabolic Factors in Cataract Production

G. W. Bartelmez: Menstruation

W. G. MacCallum: Pathological Physiology of the Prostate

Carl Voegtlin: Biochemistry of Malignant Tissues

Charles Huggins: The Composition of Bone and the Function of the Bone Cell

John H. Northrop: The Formation of Enzymes

Wm. E. Anderson and H. H. Williams: The Rôle of Fat in the Diet

V. E. Henderson and M. H. Roepke: Drugs Affecting Parasympathetic Nerves

F. R. Winton: Physical Factors Involved in the Activities of the Mammalian Kidney

Arthur G. Bills: Fatigue in Mental Work

Leslie Hellerman: Reversible Inactivations of Certain Hydrolytic Enzymes

Fred C. Koch: The Male Sex Hormones

Selig Hecht: Rods, Cones, and the Chemical Basis of Vision

Raphael Isaacs: Formation and Destruction of Red Blood Cells

Samuel R. M. Reynolds: The Nature of Uterine Contractility. A Survey of Different Trends

G. L. Brown: Transmission at Nerve Endings by Acetylcholine

A. Rosenblueth: The Transmission of Sympathetic Nerve Impulses

J. C. Eccles: Synaptic and Neuromuscular Transmission

A. E. Severinghaus: Cellular Changes in the Anterior Hypophysis with Special Reference to its Secretory Activities

Wm. Bloom: Cellular Differentiation and Tissue Culture

Hugo Krueger: The Action of Morphine on the Digestive Tract

Tentative Contents of Volume 18, 1938

E. E. Nelson and H. O. Calvery: The Present Status of the Ergot Problem

H. K. Hartline: Electrical Studies of Visual Mechanisms

Sarah S. Tower: Degeneration in Skeletal Muscle

J. R. Murlin: Some New Factors of Energy Metabolism

D. W. Bronk: Unitary Analysis of Reflex Activity

P. Bard: Functions of the Hypothalamus

H. S. Liddell: Recent Contributions to the Physiology of the Conditioned Reflex

W. H. Chambers: Undernutrition and Carbohydrate Metabolism

H. H. Woollard: Specificity in Structure and Function of Nerve Endings of the Skin

Helen Tredway Graham: The Significance of the Potentials Manifested During Nervous Activity

W. C. Rose: Nutritive Significance of Amino Acids

Phyllis Tookey Kerridge: Physiology of Hearing and Speech

A. Baird Hastings: Electrolyte Equilibria in the Blood and Body Fluids

F. Gowland Hopkins: Glutathione

C. P. Richter: The Psycho-Galvanic Reflex

E. S. G. Barron: Cellular Respiration

R. K. Burns: Experimental Transformations of Sex in Vertebrates

J. J. Morton and W. J. M. Scott: Sympathetic Vasoconstriction in Normal and Pathological Arteries of the Extremities

Tinsley R. Harrison: Arterial and Venous Pressure Factors in Circulatory Failure

Franklin F. Snyder: Causes of Onset of Labor.

J. H. Burn: Sympathetic Vasodilator Nerves.

Eliot R. Clark: Anastomoses between Arteries and Veins

M. H. Seevers and R. W. Waters: The Pharmacology of Anesthetic Gases

Thimann and Koepfli: Plant Growth Hormones

H. C. Bradley: Autolysis and Atrophy

W. H. Newton: Endocrine Functions of the Placenta

W. H. Taliaferro: Acquired Immunity to Worm Parasites

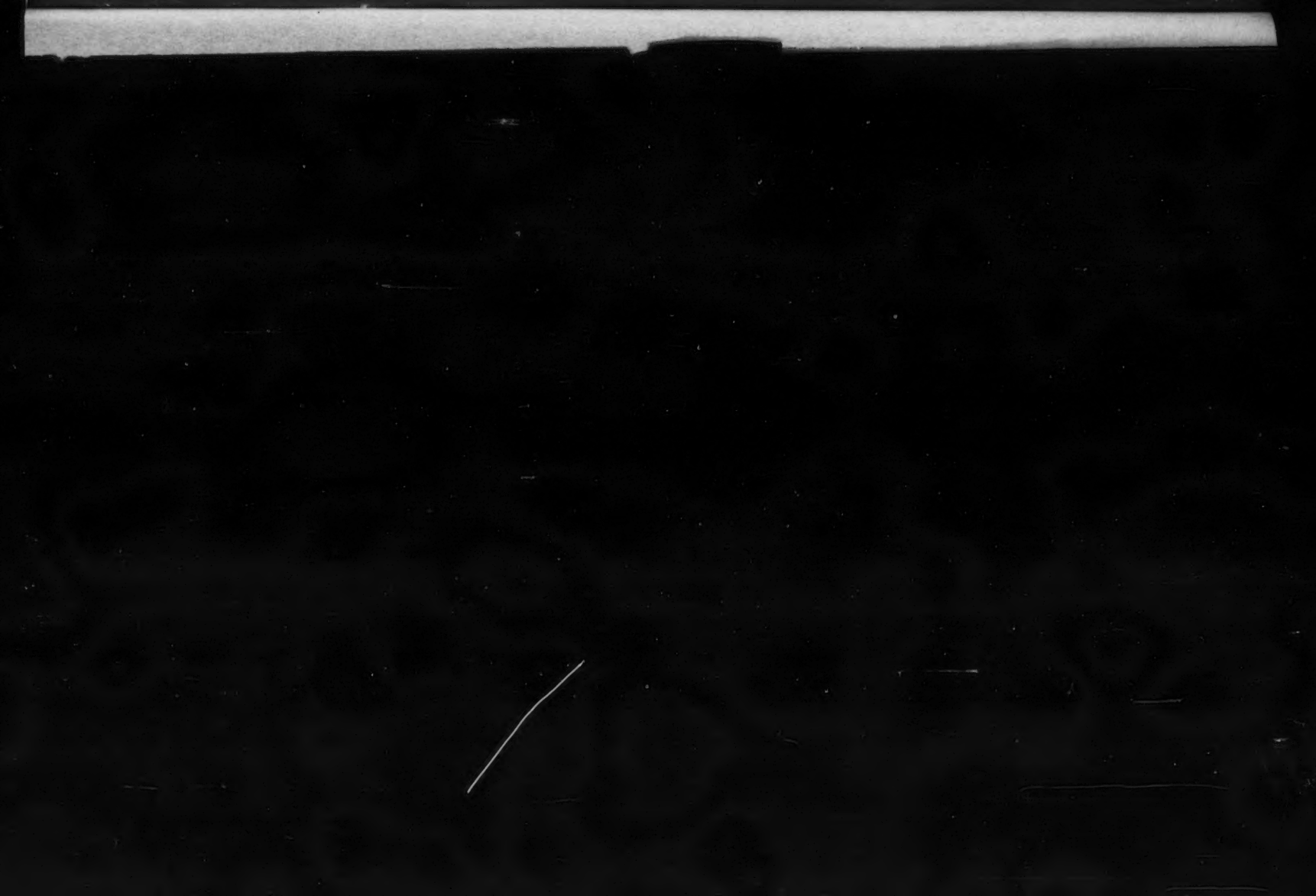
F. C. McLean: Application of the Law of Mass Action to Biological Problems

Valy Menkin: The Rôle of Inflammation in Immunity

H. M. Scott and R. M. Conrad: The Formation of the Avian Egg

E. J. King: Physiological and Pathological Effects of Silica

Stanley Cobb: Speech Abnormalities, especially Stuttering

Ann S. Minot: Physiological Action of Minute Quantities of Lead

Martin Silberberg: The Causes and Mechanism of Thrombosis